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Exploring the Biotechnological Applications of Halophilic Archaea

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The phylogenetic diversity of microorganisms living at high salt concentrations is surprising and great as well. Halophiles are found in each of the three domains: Archaea, Bacteria, and Eucarya. Many archaea colonize extreme environments. They include hyperthermophiles, sulfur-metabolizing thermophiles, extreme halophiles and methanogens. Because extremophilic microorganisms have unusual properties, they are a potentially valuable resource in the development of novel biotechnological processes. Despite extensive research, however, there are few existing industrial applications of either archaeal biomass or archaeal products in centuries-old processes. Hence this review summarizes current knowledge about the biotechnological uses of archaea and archaeal products with special attention to potential applications that are the subject of current experimental evaluation which are of key importance for the development of new biotechnological tools.

Key words: Archaea, Hypersaline environment, Biotechnological application, Phylogenetic analysis, *Crenarchaeota*, *Euryarchaeota*, *Korarachaeota*, *Nanoarchaeota* and *Thaumarchaeota*.

Studying extremophiles such as halophiles on Earth may provide insights helpful in our search for life elsewhere in the universe[1].

The Domain Archaea was not recognized as a major domain of life until quite recently. In the late 1970's Dr. Carl Woese and his colleagues at the University of Illinois, while studying relationships among the prokaryotes using DNA sequences, discovered an entirely new group of organisms- the Archaea. Those bacteria that lived at high temperatures or produced methane were clustered together as a group well away from the usual bacteria and the eukaryotes.Because of the vast differences in the genetic makeup, Dr. Carl Woese proposed that life be divided into three domains: Eukaryota, Eubacteria, and Archaebacteria. He later decided that the term

Archaebacteria was a misnomer, and shortened it to Archaea. The classification of these three primary groups was based on base-sequence studies of 16S and 18S ribosomal RNA (rRNA) molecules (Fig. 1).

The word archaea comes from the Ancient Greek αρχαια, meaning "ancient things"[3]. It is generally accepted that the Archaea diverged earliest from the common ancestor and are therefore regarded as the most primitive group of organisms. At first, only the methanogens were placed in the new domain and the Archaea were seen as extremophiles that exist only in habitats such as hot springs and salt lakes. At the end of 20th century, microbiologists realized that the Archaea are a large and diverse group of organisms, widely distributed in nature and are also common in less extreme habitats, such as soils and oceans[4]. Most

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of the Archaea are highly adapted to cope with extreme chemical and/or physical environments (temperature, pH, salinity, etc.) and the group can conveniently be divided into hyperthermophiles, halophiles and methanogens. Despite outward appearances however, the Archaea are more closely biochemically related to the Eukarya than to the Eubacteria [5].Thus today, the Archaea have come to be recognized as a domain that evolved under the conditions of the primitive earth, namely, high temperature, anaerobic atmosphere and high salinity[6].

Phylogenetic overview of the Archaea

The domain *Archaea* is divided into five phylogenetically distinct phyla named as *Crenarchaeota*, *Euryarchaeota*, *Korarachaeota*, *Nanoarchaeota* and *Thaumarchaeota* (Fig.2)[7,8].

Crenarchaeota

The term "cren" means spring or fount and expresses the resemblance of this phenotype to the ancestor of the domain *Archaea*. The Kingdom Crenarchaeota contains organisms that live in very hot and very cold environments. Most cultured Crenarchaeotes are hyperthermophiles. Hyperthermophilic archaea have been isolated from geothermally heated soils or wastes containing elemental sulphur and sulphides. The phylum comprises an important number of uncultured groups from marine plankton, freshwater and soil samples[9-13].

Euryarchaeota

The Kingdom Euryarchaeota spans a broader ecological range and includes hyperthermophiles, methanogens, halophiles and thermophilic methanogens. There also exists a large group of as yet uncultured marine euryarchaeotes. Methanogens are obligate anaerobes. These organisms thrive in anaerobic environments including marine and fresh-water sediments, bogs and deep soils, intestinal tracts of animals, and sewage treatment facilities. Five major groups are known within this phylum: obligate anaerobic methanogens, the hyperthermophilic sulfate reducers, the

Table 1. Categories of Halophilic microorganisms[30]

Category	Salt concentration (M)	
	Range	Optimum
Non-halophile	0-1.0	<0.2
Slight halophile	0.2-2.0	0.2-0.5
Moderate halophile	0.4-3.4	0.5-2.0
Borderline extreme halophile	1.4-4.0	2.0-3.0
Extreme halophile	2.0-5.2	>3.0
Halotolerant	0->1.0	<0.2
Haloversatile	0->3.0	0.2-0.5

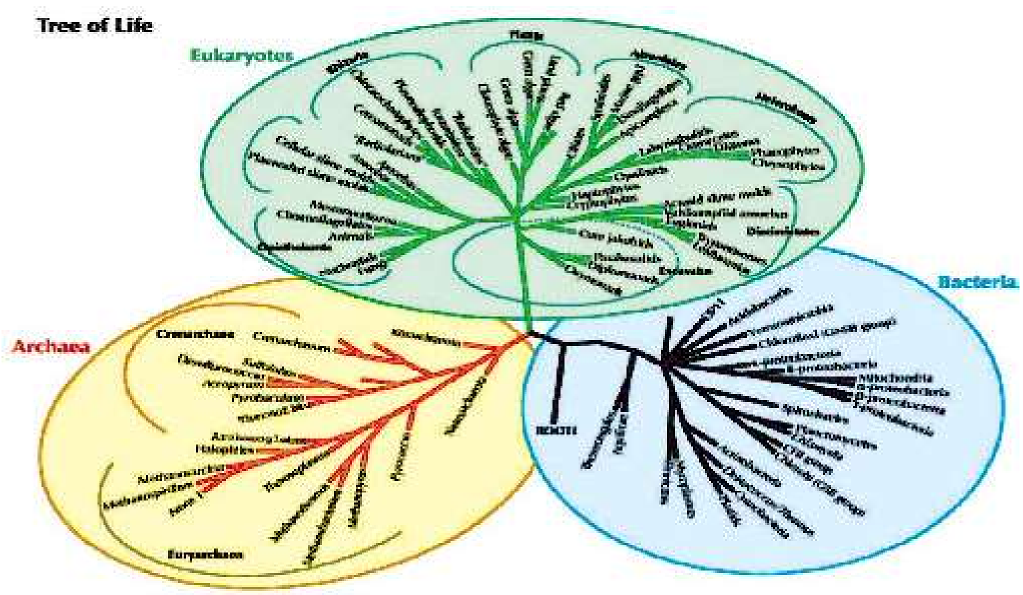

Fig. 1. Universal phylogenetic tree showing the domains of Bacteria, Eukarya and Archaea[2].

Thermoplasma group, the *Thermococcus* – *pyrococcus* group [14, 15] and the extreme halophiles.

Extremely halophilic archaea are a diverse group of prokaryotes that inhabit highly saline environments, such as solar salt evaporation ponds and natural salt lakes, or artificial saline habitats, such as the surfaces of heavily salted foods like certain fish and meats. Such habitats are often called hypersaline. Extreme halophiles are for the most part obligate aerobes. These organisms require high salt concentrations for growth, in some cases near saturation[16].

Korarcheota

The Korarchaeota were originally discovered form 16S rRNA gene sampling of an iron- and sulphur-rich Yellow Stone hot spring, Obsidian Pool. Korarchaeota a Kindom of hyperthermophilic Aarchaea that branches close to the archaeal root, and for this reason their biological properties may reveal interesting feature of ancient organisms. Although cultures of representatives of this group have now been obtained, little is yet known about them except that they are obvious hyper thermophiles growing optimally at 85°C[16].

Nanaoarcheota

Nanoarchaeota has been recently discovered as a group of Archaea currently having only one representative, *Nanoarchaeum equitans*. *Nanoarchaeum equitans* is a species of tiny microbe discovered in 2002 in a hydrothermal vent off the coast of Iceland. It is a thermophile growing in temperatures approaching boiling. *Nanoarchaeum* appears to be an obligatory symbiont on the archaeon *Ignicoccus* [17]. Its cells are only 400 nm in diameter making it the next smallest known living organism, excepting possibly nanobacteria and nanobes. Initial examination of single-stranded ribosomal RNA indicated a considerable difference between this group and the existing Kingdoms Crearchaeota and Euryarchaeota.

Studies related to open reading frames, however, have suggested that the initial sample of ribosomal RNA was biased and *Nanoarchaeum* actually belongs to Euryarchaeota[18].

The Habitat of Archaea

The methanogenic Archaea live in terminal niches in the third step of an anaerobic food chain, where organic matter is anaerobically

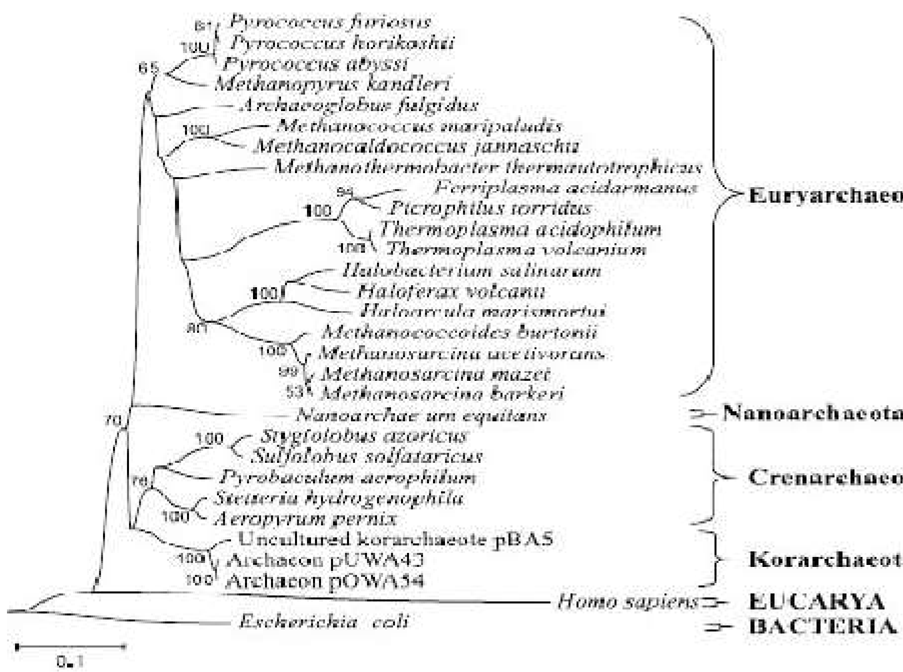

Fig. 2. Phylogenetic tree of 16S rRNA genes from archaea with genomes representing the four archaeal groups (Nanoarchaeota, Korarchaeota, Crenarchaeota and Euryarchaeota)[7]

decomposed. Methanogens have been isolated from freshwater and marine sediments, sewage sludge, soil and the intestinal tract of animals and humans. They are found in the heartwood of trees and as ecto- and endosymbionts of protozoa. In addition, geothermal heated areas are habitats of the extremely thermophilic methanogens. The extreme halophiles occur in nature in concentrated brines of marine salterns and extremely saline lakes throughout the world. Habitats are the Great Salt Lake, the Dead Sea and alkaline saline lakes such as Wadi Natrun (Egypt) and Lake Magadi (Kenya). The extremely thermophilic sulfur metabolizers occur in nature in continental and submarine volcanic areas as well as in artificial habitats such as geothermally heated power plants. Many species have been isolated from continental solfatara fields in Iceland and Yellowstone National Park or from marine hydrothermal systems near the island of Vulcano or the East Pacific Rise.

Archaeal characteristics

Several features set the Archaea apart, The halobacteria can be unequivocally distinguished from other extremely halophilic prokaryotes by their archaeal characteristics, particularly the possession of ether linked phosphoglycerides[19] (Fig. 3). The lipids of all halobacteria examined to date contain phytanyl ether analogs of phosphatidyl glycerol and phosphatidly glycerol sulphate methyl ester. Many strains also contain phosphatidyl glycerol sulphate (PGS). All halobacteria have diphytanyl (C20C20) glycerol ether core lipids, but some strains have additional phytanyl-sesterterpanyl (C20C25) glycerol ether core lipids[20, 21] and certain strains of haloalkaliphiles have di-sesterterpanyl (C25C25) glycerol ether lipids[22]. These differences may be an adaptation to extreme environments[23]. Archaeal organisms also have flagella that are notably different in composition and development from the flagella of bacteria. Individual archaea cells range from 0.1 to over 15 ìm in diameter, and some form aggregates or filaments up to 200 ìm in length. They occur in various shapes, such as spherical, rod-shaped, spiral, lobed, or rectangular, and they also exhibit a variety of different types of metabolism. Archaeal and bacterial metabolic genes share common evolutionary aspects[24]. However, the transcriptional and translational machinary of Archaea is much more similar to Eucarya than Bacteria[25].For instance, archaean translation uses eukaryotic initiation and elongation factors, and their transcription involves TATA-binding proteins and TFIIB[26].

Halophiles

Hypersaline environments

Hypersaline habitats are common throughout the world, but extremely hypersaline habitats are rather rare. Most such environments are in hot, dry areas of the world. Salt lakes can vary considerably in ionic composition. The predominant ions in a hyper saline lake depend to a major extent on the surrounding topography, geology, and general climatic conditions.

Though the oceans are, by far, the largest saline body of water, hypersaline environments

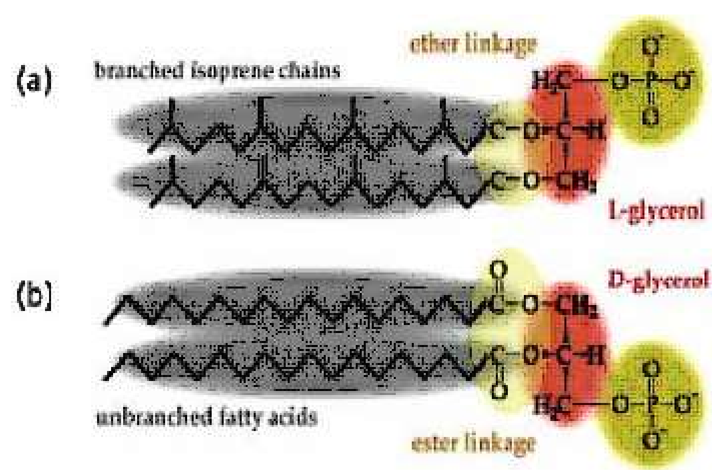

Fig. 3. Cell membrane of (a) Archaea; (b) Bacteria[27]

are generally defined as those containing salt concentrations in excess of sea water (3.5% total dissolved salts). Many hypersaline bodies derive from the evaporation of sea water and are called thalassic. A great diversity of microbial life is observed in thalassic brine from marine salinity up to about 3– 3.5 molL21 NaCl, at which point only a few extreme halophiles can grow, e.g. Halobacterium, Dunaliella, and a few bacterial species.

Athalassic waters are those in which the salts are of nonmarine proportion, found for example after the concentration of sea water leads to precipitation of NaCl, leaving a high concentration of potassium and magnesium salts and in which the pH is relatively low (around 6.0) [28]. A prime example is the Dead Sea, a lake in which the concentration of divalent cations exceeds that of monovalent cations and in which the pH is relatively low (around 6.0), the brines of the Red Sea and lakes of the Atacama Desert, Northern Chile. Even such a hostile environment periodically supports dense microbial blooms[29].Microbial life has adapted to environments that combine high salt concentrations with extremely high pH values.

Halophilic and halotolerant microorganisms can be found in each of the three domains of life: Archaea, Eubacteria and Eukarya. However, there is a continuum of lower, optimum, and maximal salt concentrations for growth and therefore, all classifications of microorganisms according to their requirement for salt and tolerance toward salt are to a large extent arbitrary (table 1).

Adaptation to saline environments

Microorganisms living in high salt environment generally adopt one of the two stratergies either “salt-in-cytoplasm mechanism” or “Compatible solute adaptations” to prevent the lost of cytoplasmic water and to established osmotic equilibrium across their cell membranes[31].

Salt in cytoplasm adaptation

Exrtrmely halophilc archaea and anaerobic halophilic bacteria uses this stratergy. This involves First accumulation of K+ and Cl ions and excludes Na+ to maintain osmotic balance. Whereas bacteria accumulates Na+ rather than K+. As a consequence of the high salt, intracellular components (e.g. proteins, nucleic acids and cofactors) require the protection from the denaturing effect of salt. The most common protective mechanism is the presence of excess negative charges on their exterior surfaces of the protein and are involved in the formation of stabilizing salt bridges or in attracting water and salt to form a strong hydreation shell e.g.malate dehydrogenase from haloarcula marismortui has 20m% excess of acidic residue over basic amino acid residue . Due to the adaptation mechanisms in the shells they have an obligate requriment of high salt concentration.

Compatible solute adaptation

They accumulate small organic and osmotically active molecules referred to as compatible solute. This compound can be synthesized denovo or imported from the surrounding medium. A large range of compatible solutes have been indentified in broad range of halophiles ranging from glycerol and other sugar alcohols, amino acids, and derivatives such as glycine, betaine and ectoine (2-methyl- 1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) and its 5-hydroxy derivatives, to simple sugars such as sucrose and trehalose [32]. All this molecules are polar, highly soluble and un charged or zwitterionic at physiological pH values. They are strong water structure formers and as such are probably excluded from the hydration shell of proteins(preferential exclusion), and therefore exert a stabilizing effect without interfering directly with the structure of the protein.

Membrane

To protect the membrane most halophilic archea posses S-layer consisting of sulphated glycoproteins,which surrounds the cytoplasmic membrane. The sulphate group confer a negative charge to the S layer and possibly provides structural integrity at high ionic concentration. In addition, archeal ether lipids have been shown to be more stable at high salt concentrations (up to 5M) compare to estar lipids founds in the membranes of bacteria.

Halophilic archaea

Taxonomy and Salient features

Halophilic archaea are classified under Domain: Archaea, Class: Halobacteria, Order: Halobacteriales and Family: Halobacteriaceae. The family *Halobacteriaceae* contains 27 genera (*Haladaptatus*, *Halalklicoccus*, *Haloalcalophilium*, *Haloarcula*, *Halobacterium*, *Halobaculum*, *Halobiforma*, *Halococcus*, *Haloferax*, *Halogeometricum*, *Halomicrobium*, *Halopiger*, *Haloplanus*, *Haloquadra*, *Halorhabdus*, *Halorubrum*, *Halosarcina*, *Halosimplex*, *Haloterrigena*, *Halovivax*, *Natrialba*, *Natrinema*, *Natronobacteium*, *Natronococcus*, *Natranolimnobius*, *Natronomonas*, *Natronorubrum*) and 96 species [33]. Description of the properties of these genera can be found in the original articles in which the establishment of the genera was proposed. The second edition of Bergey's Manual of Systematic Bacteriology[34] also provides much useful information. Recently, the extent of the family has been undergoing rapid expansion, with the description (as of June 2007) of five new genera and 20 novel species since 2006. This expansion has been due not only to the identification of novel taxa isolated from well – known hypersaline environments, such as the genera *Halovivax*, *Halostagnicola*, *Haloplanus* and *Haloquadratum* [35-37] but also to the recognition that members of the *Halobacteriales* can grown within saline microniches in non-saline environments at relatively low salt concentrations [38, 39].In addition, recent studies have reported the isolation of novel halobacterial strains [40, 41] or the detection of novel *Halobacteriales*-affiliated 16S rRNA gene sequences from moderate to low salinity system[42-44].

Resting stages are not known, although there are reports of structures referred to as halocysts in some strains [45, 46].They are non-motile or motile by tufts of flagella and stain gram negative or gram positive. Strains are aerobic or facultative anaerobic with or without nitrate and some have facultative mode of growth on arginine[47-49].Colonies of most strains possess various shades of red due to the presence of C50 carotenoids (bacterioruberins) that impart red or pink coloration to mass developments in the natural environment. These carotenoid pigments have been shown to protect the cell against photooxidative damage [50].Retinal-based pigments capable of producing light-dependent movements of ions across the cell membrane or that functioning as photosensors are present in many strains. One of these pigments bacteriorhodopsin, acts as a proton pump driven by light energy[51, 52].Some strains contain gas valcuoles, which make colonies appear pink or even white on agar media[53].

They are chemoorganotrophic, using amino acids or carbohydrates as carbon source. Occur ubiquitously in nature where the salt concentration is high like in salt lakes, soda lakes and salterns. They are common in crude solar salts and proteinaceous products (fish and hides) heavily salted with solar salt[54, 55].Optimum growth temperature lies between 35-50°C. Generation times of haloarcheal strains commonly fall in the range of 3-6 h in complex media depending on the isolate, whereas that in natural populations in brines range between 54-120 h[56]. Many strains harbour large plasmids >100 Kb. The mol% G + C of the DNA is 59-71 (major component) and 51-59 (minor component).

Biotechnological potential of halophiles

The unusual qualities of the Archaea have sparked a broad interest in potential biotechnical applications. Although Halophiles have been known for several years, the search has intensified in the last decades for two main reasons. Some applications are centuries -old, and existed long before microbiological aspects of the processes were understood. First, the range of conditions under which life can exist is now known to be much broader than previously thought, and this has led to the exploration of many hitherto uninvestigated habitats. Second, it is now recognized that attributes of organism adapted to extreme environments have the potential to serve a wide array of industrial purposes [57]. Halophilic Archaea offer important insights into the biology and evolution of many organisms and also hold the promise of providing valuable molecules for biotechnological use in industry.

Extremozymes from Halophiles

Based on the unique stability of archaeal enzymes at high temperature, pH and extreme salt concentration, they are expected to be a very powerful tool in industrial biotransformation processes that run at harsh conditions. Particularly Halophilic archaea are the most likely source of such enzymes because not only are their enzymes salt tolerant, but also thermostable[58].This is particularly true of their enzymes (called extremozymes), such as DNAases, lipases, amylases, gelatinases, xylanases, cellulases and proteases, capable of functioning under high salt conditions at which other proteins usually precipitate or denature.

α-Amylases

Amylases are a class of hydrolases which catalyse the degradation of starch polymers to produce dextrins and different gluco-oligosaccharides of variable lengths.Amylases are widely employed in different biotechnological applications including the food industry where theyare used extensively in bread and baking industry to improve the volume of dough, colour and crumb softness. Amylases are also applied in detergents to promote stain removal and are utilise in the paper and pulp industry for the modification of starches for coated paper [59]. These enzymes generally display broad pH optima and stability and they remain active at temperatures above 50°C[60].

The stability of these enzymes at extremes of pH and NaCl, as well as their ability to function optimally at elevated temperatures make them attractive candidates for hydrolysis of starch in industrial processes which are commonly performed at low water activity such as the production of syrups and also in the treatment of saline water or waste water solutions containing starch residues in the presence of high salt[61].

Proteases

Microbial proteases are one of the most extensively studied enzymes and they are widely applied in industrial processes. They are commonly used as additives in laundry detergents, food processing, pharmaceuticals, leather and diagnostic reagents, waste management as well as silver recovery[62, 63]. These enzymes display optimal activity in the presence of NaCl and maintain stability over a wide pH range (pH 5-10). In addition, the enzymes were active at temperatures of 40 – 75°C [64].

Xylanases

Xylanases play a vital role in the degradation of xylan. They are widely used in the baking industry to improve the properties of dough, and also for the past two decades the potential use of xylanases in biobleaching of paper and pulp has been growing perpetually[65].Some of these enzymes display stability at wide pH (6-11), remain active at temperatures above 60°C and may display an absolute requirement for NaCl[66-68].

Cellulases

Cellulases are mainly applied in textile industry for biopolishing of fabrics and production of stonewashed denims, as well as in laundry detergents for fabric softening and brightening[69].Interest in cellulases is also increasing in the production of bioethanol as the enzymes are used to hydrolyse pretreated cellulosic materials to fermentable sugars[70].The enzymes were reported to be thermostable, halostable and alkalostable, making them ideal candidates for various industrial applications.

Esterases and lipases

In the field of biotechnology, esterases are receiving increasing attention because of their application in organic biosynthesis. In aqueous solution, esterases catalyze the hydrolytic cleavage of esters to form the constituent acid and alcohol whereas, in organic solutions, the transesterification reaction is promoted. Both the reactants and the products of transesterification are usually highly soluble in the organic phase and the reactants may even form the organic phase themselves. The archaeal enzyme is the most thermostable (a half-life of 50 min at 126ºC) and thermoactive (optimum temperature of 100ºC) esterase known to date[71].

Bacteriorhodopsin from Halophiles

The halophilic archaeon makes use of light for both energy and sensory transduction by exploiting a family of light-sensitive proteins. The archaeal rhodopsin, a 26.5 kDa protein, has a transmembrane domain of seven helical protein segments which photons are captured via a retinal chromophor[72]. 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, Optical switches and photocurrent generators in bioelectronics and volumetric and associative optical memories[73].

Archaeosomes of Halophiles

Liposomes are lipid-bilayer bounded vesicles that can be used as a delivery vehicle for certain vaccines, enzymes, drugs and cosmetic to specific target sites in the body. The term “archaeosome” was introduced by Sprott and co-workers to describe liposomes made with ether lipids that are unique to the Archaea domain confering considerable stability on liposomal vesicles[74]. Extensive mouse model studies involving intravenous, oral and subcutaneous administration of archaeosomes demonstrated that archaeosomes are safety molecules and they are not toxic[75].

Archaeal S-layer

Like other elements of the cell envelope, S-layers play an important part in interactions of the microbial cell with the environment[76]. It was demonstrated that archaeal S-layers have been shown to be excellent patterning structures in molecular nanotechnology and biomineralization due to their high molecular order, high binding capacity and ability to recrystallize with perfect uniformity on solid surfaces, at the air:water interface and on lipid films[76].

Compatible solutes from Halophiles

Compatible solutes like ectoine and hydroxyectoine produced by some species of halophiles e.g. *Halomonas elongate KS3* used as moisturizers in cosmetics for aged, dry or irritated skin. Ectoine also has promising application as stabilizer in polymerase chain reaction[77, 78].

Exopolysaccharide from Halophiles

Bacterial extracellular polysaccharides have found different applications as gelling agents and emulsifiers, used in microbially enhanced oil recovery and they also act as a mobility controllers of petroleum. Several halophilic microorganisms e.g *Halobacterium salinarum, Haloferax volcanii and Halobacterium distributum* produce such exopolysaccharides in copious amounts, and therefore their commercial exploitation has been considered[79]. The sulfated acidic heteropoly-saccharide of *Haloferax* species has a high viscosity at low concentrations, its rheological properties are excellent and it is resistant to extremes of pH and temperature. It was therefore proposed to explore its use to enhance oil recovery from low productivity oil wells.

Biosurfactant from Halophiles

The archaeal membrane lipids may act as surfactants, improving the oil carrying properties of the water and remediation of oil polluted saline environments. Among the halophilic representatives of the Bacteria, the *Halomonas* species (*H. maura*, *H. eurihalina*) shows considerable promise as a producer of large amounts of an extracellular polyanionic polysaccharide, a potent emulsifying agent that exhibits a pseudoplastic behaviour. The *H. maura* exopolysaccharide ('mauran') has also been shown to be an immunomodulator[80].

Biofuels from halophiles

In times in which fossil fuels are getting depleted and the world is searching for alternative sources of energy, biofuel is a fashionable alternative. Although halophilicmicroorganisms may not be the most obvious source from which such fuels may be commercially produced, they still may be of interest. The hal ophilic alga *Dunaliella*, act as a potential source of glycerol production, may also be considered as the raw material for biofuel production [81-83].

Exopolymer from Halophiles

Exopolymer produced by poly (γ-D-glutamic acid) (PGA) used as a biodegradable thickner, humectants, sustained release material, or drug carrier in the food or pharmaceutical industry [84, 85].

Poly-β-hydroxyalkanoate production by halophilic bacteria

Poly-β-hydroxyalkanoate (PHA), a polymer containing β-hydroxybutyrate and β-hydroxyvalerate units, is accumulated by many prokaryotes, Bacteria as well as halophilic Archaea, as a storage polymer. It is used for the production of biodegradable plastics ('biological polyesters') with properties resembling that of polypropylene. The technology for the manufacture of poly-â-hydroxyalkanoate- derived plastics (Biopol®) was developed by ICI in the UK, using polymer produced by *Cupravidus*.

Another halophilic candidate for PHA production is *Halomonas boliviensis*, *Haloferax mediteranei, Haloferax* and *Halomonas* spp.[86, 87] that have potential as industrial producers of PHA, no attempts have yet been made to use these organisms for the commercial production of biologically degradable plastics. Such archaeal producer of the compound may have distinct advantages[88-91].

γ- Linoleic acid, β-carotene, cell extracts and Carotenoids from Halophiles

γ- Linoleic acid, β-carotene and cell extracts of Halophilic archaea is in high demand as an antioxidant, as a source of pro-vitamin A (retinol) and as a food colouring agent. Its antioxidant activities make it popular for use in health food dietary supplements, food colouring and feedstock [92-94] Carotenoids from are used in the food industry as food – coloring agents and as multivitamin preparation and additives in health food products, precursor of vitamin A, important additive in cosmetics[95].

Treatment of saline wastewaters using Halophilic microorganisms

For the biological treatment of industrial wastewaters with salt concentrations up to 10%, such as the brines generated by the pickling industry, aerobic treatment systems have been developed based on aerated percolators or rotating discs to improve aeration and mixing[96-107]. Most systems described below exist as laboratory-scale models only. Such simulations generally have shown satisfactory results at salt concentrations up to around 6%, but at higher salinities the systems performed less well. In some studies a culture of *Halobacterium salinarum* was added to improve degradation [96, 99]. Anaerobic biodegradation processes have also been proposed for the treatment of saline and hypersaline wastewater. To remove nitrate from brines, a membrane bioreactor was constructed based on denitrification by the archaeon *Haloferax denitrificans* [108], and use of *Haloferax mediterranei* has been proposed for bioremediation of nitrate and nitrite in saline groundwater in coastal areas [109].The halophilic fermentative bacterium *Halanaerobium lacusrosei* was successfully used in an anaerobic packed bed reactor operating at salt concentrations up to 10% [110,111].

Halophiles and fermented foods

Large amounts of salt are used in the preparation of certain types of traditionally fermented foods. Such salty food products are especially popular in the Far-East. Examples are 'jeotgal', traditional Korean fermented seafood, the Japanese 'fugunoko nukazuke' prepared by fermentation of salted puffer fish ovaries in rice bran, and 'nam-pla', a Thai fish sauce. The latter product is made by adding two parts of fish and one part of marine salts. The mixture is covered with concentrated brine (25–30% NaCl) and left to ferment for about a year.The first halophilic archaeon obtained from Thaifish sauce (nam pla) was an isolate resembling*Halobacterium salinarum*[55], and two new species,

Halococcus thailandensis and *Natrinema gari, were* recently isolated[112, 113]. *Halalkalicoccus jeotgali* is a novel isolate obtained from shrimp jeotgal [114]. Archaeal proteases of halophilic Archaea probably take part in the fermentation process, and the Archaea may also contribute to the aroma of the sauce.

Concluding remarks

While substantial progress has been realized of late, understanding of the biochemistry, genetics and physiology of Archaea is only just beginning. Accordingly, despite their enormous economic potential, the applied potential of Halophilic archaea remains largely unrealized. With continued basic research, development of appropriate molecular tools as well as better insight into structure–function principles, a new battery of archaeal information will become available to meet the growing industrial and biotechnological interest.

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