

## Diversity of Haloalkaliphiles from Hypersaline environment- A Review

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### Abstract:

Haloalkaliphilic bacteria are organisms which thrive in both high salt concentration and high pH habitats, such as soda lakes, soda deserts and saline and alkaline environments. These microorganisms are distributed among diverse groups in the domains bacteria, archaea and eukaryotes. Special mechanisms for adapt to both high pH and high salt concentration make the haloalkaliphiles more attractive for basic type of research and for applications in industrial biotechnology viz the production of valuable enzymes, pigments, compatible solutes, biodegradation and exo-polysaccharides. The aim of this review is to provide diversity of these organisms from such various extreme environments those are distributed worldwide to provide database for discussion to current and future biotechnological and environmental aspects.

**Keywords:** Diversity, extreme environments, haloalkaliphiles, soda lake, industrial biotechnology

### Introduction:

Extreme environments are widely distributed across the world which is exposed to one or more environmental parameters like temperature, salinity, pH, osmolarity or pressure which showing values close to the limit of life. The organisms thrive in such habitat known as extremophiles; in addition, these are normally polyextremophiles which have ability to tolerate two or more extreme conditions, like haloalkaliphiles, halothermophiles and

alkalithermophiles. Haloalkaliphiles are organisms that require high salinity (3-30%) and an alkaline pH (pH 9-13) for their growth. Haloalkaliphiles have been reported from a number of environments such as saline-soda lakes, hypersaline alkaline soils, salt mines, marine environments, brine water and salt

marshes(1,2). Haloalkaliphiles usually use small organic molecules like ectoine, betaine, proline and intracellular enzymes required maintaining their osmotic balance and pH

ranges to survive under extreme saline and alkaline environments(3).

Haloalkaliphiles have a wide range of industrial applications in detergent industry, food stuffs, paper and pulp and pharmaceuticals industry also used in production of pigments, compatible solutes, biofuel, biodegradation and exopolysaccharides(4,5). The isolation, characterizations, diversity and application of haloalkaliphiles from different environments has been studied by various groups so the aim of this review is to offer a short, but comprehensive report on the diversity of important haloalkaliphilic microbes thriving in extreme environments around the globe.

### Historical perspective of Haloalkaliphiles

The term Haloalkaliphile was first used by Soliman and Truper to describe a dual extremophile that possess halophilic and alkaliphilic attributes(6), until the only known examples of such organism belong to the Kingdom Euryarchaeota(7). After Soliman and Truper (1982) Koki Horikoshi also studied the Haloalkaliphiles in detail and described as alkaliphiles consist of two physiological groups of microorganisms, alkaliphiles and haloalkaliphiles. Alkaliphiles require an alkaline pH of 9 or more for their growth, whereas haloalkaliphiles the group of bacteria able to grow under alkaline conditions in the presence of salt require both an alkaline pH (>pH 9) and high salinity (up to 33 % (w/v) NaCl) (2,5).

Haloalkaliphiles are found in both natural and artificial environments in nature, they possess special adaptation mechanisms for survival in highly saline and alkaline pH in such extreme environments which include

production of high density branched chains lipids and increased content of cell wall components along with Na<sup>+</sup>/H<sup>+</sup> antiporters as internal pH homeostasis (5).

Studies on ecology, physiology, and taxonomy of haloalkaliphiles revealed an impressive microbial diversity in many saline and alkaline lakes from world. These dual properties made haloalkaliphilic organisms interesting for, basic research and for industrial applications in biotechnology (4,8). Haloalkaliphilic organisms are widely distributed along the phylogenetic tree of life forms, and significant diversity of these microorganisms from major taxonomic groups was reported by various research groups.

Haloalkaliphilic microorganisms have largely been identified and studied from worldwide various locations like Soda Lake, soda deserts, hypersaline and alkaline environments, carbonate springs, salt brines, alkaline soils, Dead Sea, saltern crystallizer ponds and places saturated with respect to sodium chloride. So far, large numbers of hyper saline alkaline environments have been studied to know their chemical composition and ecological diversity of these extremophiles (9).

The large number of haloalkaliphilic bacterial and archaeal strains depicted wide diversity, as reflected through microbiological, biochemical and molecular approaches (10-13).

During the past years special attention has been focused on the distribution of haloalkaliphiles and their diversity(14,15). Culture dependent and independent methods are usually used to investigate biochemical characters, genetic and phylogenetic diversity of

haloalkaliphiles from different extreme environments (16). Recently characterization and diversity study from hypersaline lakes of northern Egypt has been carried out by Arayas and their coworker's, they reported massive culturable aerobic haloalkaliphilic diversity from Marsa-Matrouh lake and Al-Hamra lake (17).

In the past few decades soda lakes environments has been studied in detail for existence of extremophiles by various research group. Initially haloalkaliphiles were reported as soda lake inhabitants, a large number of haloalkaliphilic bacteria and archaea were obtained from such environments. Early ecological studies related to haloalkaliphilic microorganisms were reported from the alkaline hypersaline lakes such as Wadi Natrun and Lake Magadii from Kenya (10).

Banda and their research group also studied soda lake Microbial Community with the Effects of Salinity and pH on Diversity and Distribution Pattern in the Brines of soda lake from Badain Jaran Desert, China. Banda and group found significant difference in microbial communities inhabiting the different alkali-saline lakes, both pH and salinity shaped the haloalkaliphilic community in such habitat (18). Sorokin and their research group studied extensively biogeochemical cycling occur in soda lakes and they reported diverse and novel group of haloalkaliphiles from such extreme habitat (19).

One of the noticeable features of such habitats/ lakes is their colour. The water colour of lake may appear from green to red due to the massive blooms of microorganisms (20,21).

### Diversity of Haloalkaliphiles

### Oxygenic and anoxygenic phototrophs and heterotrophs

Jannasch (1957) was the first to report about the red colour development in such lakes in which the salt concentration exceeding 200g-1 to mass development of photosynthetic purple bacteria also these bacteria are capable for the sulfate reduction in the bottom sediments and oxidation of the sulfide. He reported *Chromatium* and *Thiospirillum* purple bacteria from brine samples of lake. These lakes are most productive in the world for such organisms (20).

The photosynthetic productivity was mostly due to the dense populations of cyanobacteria. *Spirulina* sp. is usually most dominating amongst such cyanobacteria blooms. Earlier Imhoff and his co-workers carried out detailed study of lake Gabara (91.9g-1 salt) in 1978 and 1979, which is inhabited by *Spirulina* and other cyanobacteria spp. (22,23).

These cyanobacteria are the principle food of the vast flocks of Lesser Flamingo that inhabit the soda lakes. Other than *Spirulina* few additional cyanobacteria are *Ectothiorhodospira*, *Halorhodospira* and *Thiorhodospira* also relate to the primary productivity and these members are acting as a link between the S- and C- cycle of soda lakes (14).

Imhoff and Truper have reported *Ectothiorhodospira halochloris* from the Wadi Natrun Egypt, which appears in green and brown color due to bacteriochlorophyll b production, optimal salinity of lake was reported 14-27% and pH 8.1 to 9.1 (24). Other members of same genus were reported worldwide by different research groups which mainly includes

(*Ectothiorhodospiravacoulata* reported by) Imhoff et al., in 1981; Kompantseva et al., in 2005; and Upasani, in 2008 (24-26). Additionally red algae like *Dunaliella salina*, *Arthrospira platensis*, *Synechococcus cedrorum*, *Anabaena* sp. are reported from Indian soda lakes (27).

*Synechocystis salina*, *Aphanothece stagnina*, *Chamaesiphon subglobosus*, *Rhabdoderma lineare*, *Synechococcus elongatus*, *Spirulina fusiformis*, *Phormidium ambiguuum*, *Phormidium retzii*, *Oscillatoria splendida*, *Phormidium foveolarum* and *Oscillatoria limnetica* species, all these benthic cyanobacteria were reported by Dubinin and his co workers from Lake Magadi (28).

Other than cyanobacterial population existence of wide aerobic and anaerobic bacteria were reported from extreme hypersaline alkaline environments worldwide by research groups like Duckworth et al. 1996, Imhoff et al., 1979, Woese et al., 1985, Grant et al., 1990, Zavarzin et al., 1999; Mesbah et al., 2007, Sorokin et al., 2002 (10,23,29-33).

### Aerobic haloalkaliphilic bacteria

Obvious succession of microbial community in hypersaline ecosystem was revealed by observing existence of aerobic bacteria like *Bacillus*, *Halomonas* sp. and anaerobic hydrolytic bacteria *Clostridia* by various research groups. These organisms were reported as consumers of polymers synthesized by cyanobacteria (14,23).

Grant and colleagues has been done the survey regarding correlation between the aerobic heterotrophic bacteria to cyanobacteria in which bacterial numbers were constant and the dominant types were varied, such type of survey was performed on selective media (30).

Extremely diverse group of aerobic, heterotrophic and organotrophics alkaliphilic and haloalkaliphilic organisms were reported by Duckworth et al. from East African Rift Valley (10), besides comprehensive study made by him and his group to generate a report on Gram-positive bacteria of both the high G+C (Firmicutes) and low G+C (Actinobacteria) lineages from such environments (10). The Gram-positive and Gram negative bacteria reported by Duckworth and his coworkers in this study belonged to *Bacillus* genus and gamma proteobacteria respectively. Similar results were obtained worldwide using such types of habitats (29,34,35).

It was recorded that, *Bacillus* genus is dominating belonging to phylum Firmicutes and commonly present in such haloalkaline environment.

Haloalkaliphilic *Bacillus* species are the group specifically adapted to grow optimally under moderate halophilic and alkaline conditions, apart from *Haloarchaea*, *Bacillus* species are highly found in such habitat, particularly in saturation ponds, which have 15-25 % salinity. *Bacillus* is the dominant bacterial species in microbial fermentations and important participant in production of various valuable industrial products. Syed Shameer has described bioprospecting study of haloalkaliphilic *Bacillus* species from solar salterns (36).

The novel haloalkaliphilic species from *Bacilli* taxon were isolated and studied by various research groups. Some of the *Bacillus* members were isolated and grouped into new genera: *Alicyclobacillus*, *Virgibacillus*, *Brevibacillus*, *Aneurinibacillus*, *Halobacillus*, *Gracilibacillus* and *Paenibacillus* (37-38).

Similar report showed recently by Arayes and their coworker's, the massive culturable aerobic haloalkaliphilic diversity from Marsa-Matrouh lake and Al-Hamra lakes of northern Egypt, they showed haloalkaliphilic organisms diversity belonging to phylum Firmicutes and the genera like *Alkalibacillus*, *Salinicoccus*, *Gracilibacillus*, *Thalassobacillus*, *Halobacillus*, *Staphylococcus* and *Bacillus*(17).

Large evidence suggests that diverse haloalkaliphilic *Bacillus* species were isolated from various haloalkaline environments from worldwide (34,36). Recently from Indian soda lakes haloalkaliphilic *Bacilli* diversity was explored by many research groups Tambekar and Dhundale reported the phenotypic analysis of *B. flexus*, *B. cellulosityticus*, *B. pseudofirmus*, *B. clausii*, *B. krukwichiae*, *B. pumilus*, *B. lehensis*, *B. halodurans*, *B. circulans*, *B. cereus*, *B. agaradhaerens*, *B. sphaericus*, *B. fusiformis*, *B. asahii*, *B. pseudalcalophilus*, *B. okuhidensis*, and *B. gibsonii*(39).

Next to the Gram-positive the majority of Gram negative isolates cultivated belonged to gammaproteobacteria (29). One of the dominant genus Halomonads in gammaproteobacteria was reported from by various groups along with Indian Sambhar and Lonar soda lake (12,40,41). East African soda lakes(42), Hypersaline environments of China(43), solar salterns at Tacan in Korea (44)salt lake of Ras Muhammad(45).

These haloalkaliphilic halomonads have attracted researchers due to their special ability of denitrification and oxidation of thiosulfate and sulfide and they are reported by Sorokin et al. (46). Besides gammaproteobacteria the presence of other proteobacteria related to *Pseudomonas spp.*,

*Aeromonas spp.* and *vibrio* were also shown by Duckworth and their group (10).

These all lineages were later on reported world wide from different haloalkaline ecosystems such as lake Elmenteita from Kenyan Rift Valley (47), Chaka lake, China (48), soda lakes from Kenyan-Tanzanian Rift valley (49), Former soda Texcoco lake, Mexico (50), Hypersaline Mono lake, California (51,52).

### Methylotrophic haloalkaliphilic bacteria

Soda lake habitats were extensively studied for various haloalkaliphiles, from such environments the methane, methanol and methylamine utilizing organisms also reported by different groups.

Haloalkaliphilic methylotroph *Methylophaga lonarensis* bacterium was isolated from the Lonar soda lake sediments (53) and novel obligately methylotrophic, methane-oxidizing *Methylomicrobium* species was isolated by Sorokin in 2000 from a highly alkaline environment (54).

These research groups studied such soda lakes of widely different geographical sites and they isolated and reported new haloalkaliphilic species, from the soda lake like Wadi al Natrun (32), Few examples of new haloalkaliphilic species like *Halomonas mongoliensis sp. nov.* and *Halomonas kenyensis sp. nov.*, new haloalkaliphilic denitrifiers capable of reducing N<sub>2</sub>O reported by Boltianskaia et al.(55), *Alkalilimnicola ehrlichii sp. nov.*, a novel, arsenite-oxidizing haloalkaliphilic gammaproteobacterium capable of chemoautotrophic or heterotrophic growth with nitrate or oxygen as the electron acceptor showed by Hoefft and their research group (56), *Salinivibrio sharmensis* a novel haloalkaliphilic bacterium was reported from

a saline lake in Ras Mohammed Park (Egypt)(57) and Namsaraev and his group were reported haloalkaliphilic, *Marinospirillum celere* bacterium from Mono Lake(58).

### Anaerobic haloalkaliphilic bacteria

Existence of anaerobic haloalkaliphilic bacteria was reported in 1990 however systematic detailed studies were carried out 1996 onwards, Jones and his coworkers were obtained such organisms from extreme hypersaline habitats like Lake Magadi and placed them as a new genus of obligately anaerobic haloalkaliphiles using phylogenetic analysis(20) and in 1999 Zhilina, Zavarzin and his coworkers have reported soda lake anaerobes from a variety of lakes in the former USSR(31,59).

Considerable diversity of anaerobic haloalkaliphilic organisms was shown from worldwide. The diversity of anaerobic organisms from Egyptian soda lakes of the Wadi al Natrun was shown by Mesbah and colleagues(32). Haloalkaliphilic anaerobic organism like *Alkalilimnicola ehrlichii* was obtained from Mono Lake California, USA by Hoefft et al. in 2007(56)

Besides soda lakes various types of *Tindallia texcoconensis*, was reported from Texcoco, Mexico Soda lake(60), *Sulfidogens* and *Desulfitispora alkaliphila* species were reported by Sorokin and Muyzer(61).

Besides soda lake various types of haloalkaliphilic anaerobic bacteria were reported from other sources by many research groups like *Halonatronum saccharophilum* bacterium was reported by Zhilina et al. from the coastal lagoon mud of the Lake Magadi (Kenya) (59).

The occurrence of haloalkaliphilic acetogenic bacteria were observed in bottom mud of the Lake Magadi, Kenya, *Natroniella acetigena*, *Thermosyntropha lipolytica*, *Tindallia californiensis*, *Tindallia magadiensis* were reported from such soda lake environments like Mono Lake (California) by Pikuta(62,63). Recently *Proteinivorax tanatarense*, was isolated from a decaying algal bloom (64).

Besides soda lakes and soda deserts few natural and artificial ecosystems have been investigated and showed presence of various types of haloalkaliphilic bacteria. *Halomonas campisalis*, denitrifying bacterium was reported from the salt plain of Alkali Lake in Washington State (USA) (65). Arylaliphatic nitriles utilizing haloalkaliphilic *Halomonas nitrilicus* was isolated from soda soils by Chmura and their colleague in 2008 (66).

Recently haloalkaliphilic representatives of nitrifying, sulfur-oxidizing, H<sub>2</sub>-oxidizing, carboxydrotrophic and fermentative bacteria have recently been isolated from soda lakes and characterized by Sorokin et al., Two novel fermentative anaerobic haloalkaliphiles (*Natranaerovirga pectinivora* and *Natranaerovirga hydrolytica*) from soda lakes can use pectin as substrate either at moderate (*Natronoflexus pectinovorans* from the Bacteroidetes) or high salt concentration (*Natronovirga* from the Clostridiales) showed by Sorokin and his research group (67,68).

### Haloalkaline enzymes producing haloalkaliphiles

Haloalkaliphiles are useful in biotechnology as sources of novel enzymes and proteins, they have developed diverse biochemical, structural and physiological modifications which allowing the catalytic synthesis of proteins with interesting physicochemical and structural properties. The haloalkaline

enzymes that allows them to be considered as a novel alternative for use in the biotechnological industries because their polyextremophilicity, i.e. they have the capacity to be tolerate a wide range of pH and tolerate high salt concentrations.

Most of the haloalkaliphiles are exhibited protease, lipase, cellulase, amylase, gelatinase, xylanase and catalase activities which reported by many research groups among this protease producing haloalkaliphiles are dominating once.(4,13,16,26,36,40,41,64).

A few industrial applications of these enzymes have been reported however it is important to investigate applications of this haloalkaline enzymes in more biotechnological processes. Few enzyme producers are Haloalkaliphilic *Nesterenkonia spp.* was reported from various research groups, like Govender and his coworkers in 2009 and Nel and thir colleague in 2011, from Antarctic desert soil and Sun salt pan of Botswana respectively (69,70).

Other than enzyme producing ability various metabolically active and other important bioactive compounds like compatible solutes, pigments and exo-polysaccharides producing haloalkaliphiles are reported worldwide, few examples of haloalkaliphilic bacteria and archaea producing these industrially important product are listed in Table 1 (71-78).

Microorganism	Product	Reference
<i>Methylophaga lonarensis</i> MPL <sup>T</sup>	ectoine, glutamate	Antony et al., 2012 (71)

<i>Desulfonatronospira thiodismutans</i> ASO3-1 <sup>T</sup>	glycine betaine	Sorokin et al. 2011 (46)
<i>Natronococcus</i> , <i>Natronolimnobius</i> , <i>Halorubrum</i> , <i>Natronomonas</i>	biosurfactants	Selim et al. 2012 (72)
<i>Thioalkalivibrio versutus</i>	sulfur-oxidizing	Banciu et al. 2004 (84)
<i>Thioalkalimicrobium cyclicum</i> , <i>Thioalkalivibrio jannaschii</i>	sulfur-oxidizing	Sorokin et al. 2002 (33)
<i>Halomonas campaniensis</i> MCM B-1027	hydroxybutyrate-co-hydroxyvalerate (PHB-co-PHV) copolymer	Kanekar et al. 2011 (73)
<i>Natrialba magadii</i> ATCC 43099 <sup>T</sup>	protease	Tindall et al. 1980 (93), Mwatha & Grant 1993(94), Giménez et al. 2000 (74)
<i>Natronolimnobius innermongolicus</i>	protease	Selim et al. 2014 (107)
<i>Nesterenkonia spp</i>	protease, xylanase	Govender et al. 2009 (69), Nel et al. 2011 (70)
<i>Bacillus</i> sp. Vel	protease	Patel et al. 2005 (75)
<i>Clostridium alkalicellulosi</i> DSM 17461 <sup>T</sup>	cellulose, xylanase	Zhilina et al. 2005 (82)
<i>Desulfonatronum</i> spp	sulfate-reducing	Sorokin et al. 2011 (46)
<i>Desulfonatronovibrio</i> spp		
<i>Thioalkalivibrio versutus</i> strain ALJ 15	Pigments natronochrome and chloronatronochrome	Takaichi et al. 2004 (76)
<i>Halomonas</i> spp.	denitrification	Shapovalova et al. 2008 (78)
<i>Thioalkalivibrio nitratireducens</i> ALEN 2 <sup>T</sup>	denitrification	Sorokin et al. 2003 (77)
<i>Halomonas nitrilicus</i> sp	biodegradation	Chmura et al. 2008 (66)

Also few members of metabolically versatile haloalkaliphiles from halophilic habitats was reported, such as *Nitriliruptor alkaliphilus* and

methylophilic *Methylophaga sulfidovorans* (79-80).

### Sulfate-reducing and Sulfur-oxidizing haloalkaliphiles

In recent year's development of various molecular tools for identification have resulted in systematic and detailed investigation of haloalkaline ecosystems. These reports mainly include data based on 16S rRNA identification method, FAME analysis, with these tools scientist have reported diverse haloalkaliphiles and grouped them into different groups.

Sulfate-reducing bacteria is one of the major group from Soda lake habitat which explore and studied by Sorokin and his colleague and they reported novel *Desulfonatronum thioautotrophicum*, *D. thiosulfatophilum* and *D. magnus*, *Desulfonatronovibrio thiodismutans* and *Desulfonatronospira thiodismutan* and *Desulfonatrobacter acidivorans* and *Desulfobulbus alkaliphilus* from sediments of soda lakes in Kulunda Steppe (Altai, Russia)(46,81). *Desulfonatronovibrio hydrogenovorans*, *Desulfonatronum cooperatum* were reported by Zhilina et al and *Desulfonatronum lacustre* and *Desulfonatronum thiodismutans* were showed by Pikuta et al. in 2003(82,83).

Sulfur-oxidizing *Thioalkalimicrobium cyclicum* and *Thioalkalivibrio jannaschii* were isolated from Mono Lake(California) and *Thioalkalivibrio versutus* was reported from Kenyan Soda lake(33,84). The genera *Thioalkalimicrobium* and *Thioalkalivibrio* were reported from various lakes of the Kenyan Rift Valley (Bogoria, Crater lake Sonachi, Elmenteita, Nakuru and Magadi) and the low-saline Siberian soda lakes (Hadyn, Tsaidam, Low Mukei)(33).

Upasani also showed haloalkaliphilic anoxygenic phototrophic Sulfur-oxidizing bacteria in India from Sambhar Soda Lake(27). Saline soda lakes of the Central Asia (*Rhodovulum steppense*) and brackish steppe soda lakes of southern Siberia (*Rhodovulum tesquicola*) also a suitable environment for growth of haloalkaliphilic purple nonsulfur bacteria (85,86).

These all few representative members of diverse and important aerobic and anaerobic, methanogenic, haloalkaline enzyme producing, sulfate-reducing and sulfur-oxidizing haloalkaliphiles from soda lake and extreme habitats rather than these organisms, haloarchaea a distinct group also found in such environment.

### Haloalkaliphilic Archaea

The haloalkaliphilic archaea is a distinct physiological group due to their obligate alkaliphily. These extremely halophilic, aerobic archaea placed in the order Halobacteriales, family Halobacteriaceae, and class Haloarchaea that require least 1.5M NaCl for growth(87).

Assessment of a various alkaline and hyper saline lakes from different geological locations of the world, indicated that haloalkaliphilic archaea of the family Halobacteriaceae (so called "halobacteria"), are found in all such lakes and environments (88). Halobacteria are the most dominant microbial population found when hypersaline waters come up to saturation, frequently importing a red coloration to the brines because of C<sub>50</sub> carotenoids (89).

Currently the classification of this family is mainly based on three taxonomical characters those are 16S rRNA gene sequence, polar lipid composition, and DNA-DNA hybridization (90). Initially



Haloarchaea are classified solely on morphological and biochemical criteria, this group initially consists of only two original genera, *Halobacterium* and *Halococcus*, subsequently expanded to six genera(91)but further aerobic, extremely haloarchaea are classified into 28 different genera.

Haloalkaliphilic haloarchaea were assigned firstly in to the genera *Natronobacterium* and *Natronococcus* described by Tindall et al., followed a study of Lake Magadi in Kenya, after earlier reports of red halophiles at Kenyan and Egyptian alkaline hypersaline sites(6,92, 93).

Such saline soda lakes support blooms of halobacteria and harbour alkaliphilic representatives of the genera *Natronobacterium* and *Natronococcus*, *Natronomonas*, *Natrialba*, *Natronorubrum* and *Halorubrum*. Functionally, they have a specific trophic position and flourish on the organic matter concentration arising from evaporation of brine and the death of its microbial population(31). Haloalkaliphilic strains that require high pH, high salt and these organisms also exhibited very low requirements for  $Mg^{2+}$ . Several of the haloarchaeal genera referred to earlier exclusively harbor haloalkaliphilic types (often genera with Natrono pre- fixes) (93).

Haloalkaliphilic archaea have been reported from hypersaline alkaline habitats such as soda lakes, soda deserts and soda soils at many different geographical sites by various research groups, for example, Lake Magadi in Kenya(10,94,95), the Wadi Natrun in Egypt (6,96), Owens Lake in California, soda lakes in China, Inner Mongolia, and Tibet (97-102)and from soda lakesof India(27,103).

Novel archaeobacterial diverse strains were isolated and reported by many groups from

worldwide. *Natronolimnobius baerhuensis* and *Natronolimnobius innermongolicus* and *Natrialba hulunbeirensis* and *Natrialba chahannaoensis* novel haloalkaliphilic archaea were isolated from soda lakes of Inner Mongolia, China (98,101).

Novel extremely haloalkaliphilic members form hypersaline alkaline lakes from Central Asia, Egypt and USA reported by Sorokin et al. Phenotypic and physiological investigation showed clear growth pattern of these members on various substrates. Six extremely euryarchaea reported from such environments were isolated on cellulose as growth substrate and named *cellulotrophic natronoarchaea*, *Natronobiforma cellulositropha* gen. Nov., and extremely haloalkaliphilic members *Natronotalea proteinilytica* gen. nov., sp. nov. and *Longimonas haloalkaliphila* sp. nov., these two proteolytic bacterial strains from the phylum *Rhodothermaeota* were reported from sediments of hypersaline alkaline lakes in Kulunda Steppe (Altai, Russia) by Sorokin and his team in 2017 (104,105).

Following table shows published haloalkaliphilic archaeal species that have been isolated from soda lakes Table 2.

Species	Original location	Reference
<i>Natronococcus occultus</i>	Lake Magadii, Kenya	(Tindall et al. 1984)
<i>Natronococcus amylolyticus</i>	Lake Magadii, Kenya	(Kanai et al. 1995)
<i>Natronobacterium gregoryi</i>	Lake Magadii, Kenya	(Tindall et al. 1984)
<i>Natronomonas pharonis</i>	Lake Magadii, Kenya	(Tindall et al. 1984) (Kamekura et al. 1997)
<i>Natrialba magadii</i>	Lake Magadii, Kenya	(Tindall et al. 1984) (Kamekura et al. 1997)
<i>Natrialba hulunbeirensis</i>	Hulunbeir Province, Inner Mongolia	(Xu et al. 2001)
<i>Natrialba chahannaensis</i>	Lake Chahannor, Inner Mongolia	(Xu et al. 2001)
<i>Natronolimnobius baerhuensis</i>	Lake Baer, Inner Mongolia	(Itoh et al. 2005)
<i>Natronolimnobius innermongolicus</i>	Lake Baer, Inner Mongolia	(Itoh et al. 2005)
<i>Natronorubrum bangense</i>	Bange Lake, Tibet	(Xu et al. 1999)
<i>Natronorubrum tibetense</i>	Bange Lake, Tibet	(Xu et al. 1999)
<i>Halorubrum vacuolatum</i>	Lake Magadii, Kenya	(Mwatha and Grant 1993) (Kamekura et al. 1997)
<i>Halorubrum alkaliphilum</i>	Xinjiang Province, China	(Feng et al. 2005)
<i>Halorubrum luteum</i>	Lake Chahannor, Inner Mongolia	(Hu et al. 2008)
<i>Halorubrum tibetense</i>	Lake Zabuye, Tibet	(Fan et al. 2004)
<i>Halalkalicoccus tibetensis</i>	Lake Zabuye, Tibet	(Xue et al. 2005)
<i>Halobiforma nitratireducens</i>	Lake Chahannor, China	(Hezayen et al. 2002)

Table 2 Soda Lake Haloarchaea (adopted from 'Handbook of Extremophiles')

Recently a novel haloalkaliphilic archaeon *Natronobacterium texcoconense* isolated from soil of the former lake Texcoco in Mexico(106). Thermostable alkaline halophilic-protease producing *Natronolimnobius innermongolicus* WN18 was isolated from Soda lake of Wadi An-Natron, Egypt by Samy Selim and their coworkers(107).

In the genera *Natronococcus* various species were reported like *Natronococcus jeotgali*(103,108),*Natronococcus occultus*(93,103),*Natronococcus amylolyticus*(103,109)and *Natronococcus roseus*(110). Kajale and his research group work on Hypersaline Sambhar Lake for cultivation of diverse microorganisms. Large number of archaea and bacteria were isolated using different cultivation approaches; they reported *Natronococcus* and *Alkalibacillus* as

predominant groups in such extreme habitat(111). *Natronorubrum sulfidifaciens*, *Haloterrigena daqingensis* and *Halalkalicoccus tibetensis* an extremely haloalkaliphilic archaeon were reported from saline and alkaline environments of China(102, 112).

Due to the great interest in haloalkaliphilic organisms, all these members of hyper saline and alkaline environments were reported and studied by various researchers.

### Conclusion

Extensive number of cultured haloalkaliphiles has been obtained from culture dependent methods which uncovered a much more diverse haloalkaliphiles from various extreme environments from world. Future studies should attempt to isolate diverse members of the uncultured community from such extreme habitats. Using culture independent methods, phylogenic and metagenomics may apply to obtain a diverse range of the haloalkaliphilic organisms from such hypersaline environments. Metagenomics study can be used to explore the overall metabolic capacity of the microbial communities. Several metabolic processes followed by haloalkaliphiles and their byproducts have not yet been detected

Haloalkaliphiles are interesting extremophiles, hypersaline and alkaline environments explored for microbial diverse communities of haloalkaliphiles, this will contribute to our understanding of these ecosystems and can benefit in designing the applications. These ecosystems represent a valuable source of different industrial compounds with great economical potential and microbial diversity can prove to be a valuable future resource in various industrial and biotechnological processes.

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