SVOA Microbiology

ISSN: 2634-534X

ScienceVolks Open Access

Review Article

Halophilic Bacterial Diversity of Sambhar Salt Lake, Rajasthan, India

Nisha Chakravati[1], Varsha Gupta[1], Devki[1], Ravi Kant Rahi[1] and Deepesh Kumar Neelam*[1]

[1]Department of Microbiology, Faculty of Science, JECRC University, Ramchandrapura Industrial Area, Vidhani, Sitapura Extension, Jaipur, Rajasthan, India 303905

***Corresponding Author:** Dr. Deepesh Kumar Neelam, Department of Microbiology, Faculty of Science, JECRC University, Ramchandrapura Industrial Area, Vidhani, Sitapura Extension, Jaipur, Rajasthan, India 303905, ORCID Id:- 0000-0002-4693-8075*

***Received:** September 26, 2022* ***Published:** October 14, 2022*

Abstract

Saline environment contains higher salt concentration similar to sea water and have high importance in the study of microbial diversity. There are various saline lakes located all over the world such as Lonar Lake, Magadi Lake, Sambhar Lake etc. Among all these the Sambhar Lake is the largest inland lake in India which provides saline and alkaline environment. The salinity in this lake is upto 40 gms per litre makes it hypersaline in nature. The lake has great diversity of halophilic bacteria on the basis of their salt concentration as slight, moderate and mild halophiles. Only the halophilic archaea and halophilic bacteria are more habitual for the hypersaline environment. They survive in extreme saline condition by consuming energy to eliminate salt from the cytoplasm to elude the aggregation of protein. Some of the example of halophilic bacteria from recent studies is *Piscibacillus, Anabaenopsis* and *Halomonas*. This study gives an understanding about the saline environment and how the halophilic microorganisms thrive under these conditions, their diversity in Sambhar Lake and their industrial applications.

***Keywords:** Saline environment, Sambhar Lake, halophilic microbial diversity*

Introduction

Saline environments are extreme environment with the hypersaline conditions. It is of two types thalassohaline environment and athalassohaline environment conditions [1]. The environment on the Earth which met this condition are distinctive and possess the characteristics such as; neutral pH, intermediate temperature, abundant oxygen level, salinity of seawater and freshwater and pressure for few atmospheres [2]. Halophiles are the form of extremophiles that continuously existing in excessive salt concentrations. It is broadly distributed all over the world in saline conditions. They prevent the denaturation of salts and assemble inorganic ions in the cytoplasm such as (K^+, Na^+, Cl^-) in order to maintain the osmotic pressure of the environment. They include two major domains such as prokaryotic (bacteria and archaea) and eukaryotic microorganisms. Haloarchaea and halo bacteria are isolated from athalassohaline environment and thalassohaline environment [1,3]. Bacteria and archaea have hypersaline environments; they constitute extreme environmental conditions having high salinity, high and low temperature, low oxygen availability, high pH range [4]. Halophilic bacteria are classified on the basis of their salt concentrations as: slight halophiles grow optimum at 0.2-0.85M (2-5%) NaCl, moderate halophiles grow optimum at 0.85-3.4 M (5-20%) NaCl and extreme halophiles grow optimum at 3.4-5.1 M (20-30%) NaCl [5]. Some halophiles live in moderate environmental conditions such as the temperature ranges from 20- 40° C, neutral pH, water availability, salts and some macro-micronutrients which are required for mesophiles growth [6]. Osmotic pressure is regulated by adapting two strategies; first strategy involves accretion of K^+ and Cl^- ions to balance osmotic pressure in cell and second strategy involves accretion of organic solutes such as ectoine hydroxyectoine N_γ-acetyldiaminobutyrate β-glutamine, betaine, trehalose, proline to maintain osmotic balance in cell so that they can exist in saline environment [7]. Researchers report the isolation of slightly halophilic bacteria. This bacterium can accumulate 2,4,6-trinitrotoluene (TNT) which leads towards its detoxification8. The halophilic bacteria grow under saline conditions are fascinated by their physiological features, growing parameters and producing bioactive metabolites. Application of halophiles is the production of biomolecules for therapeutic use [9]. The Sambhar Lake is located in Rajasthan with the saline and alkaline medium. The diversity of halophilic bacteria in Sambhar Lake is diverse. This lake is rich in proportion of Na^+ and Cl^- ions by making hypersaline environmental conditions [10]. It was found that the *Vibrio proteolyticus* strain isolated from the Korean marine environment which produces polyhydroxyalkanoate (PHA) [11].

From wet salted hides, a moderately halophilic bacterium was isolated named, *Halomonas pellis* sp. that optimally grows at pH of 8 at 30 °C in presence of 10% NaCl [12]. It was also found that halophiles produce the class of dehalogenases and plays a major role in bioremediation [13]. The utilization of halophilic bacterial enzymes is not restricted to their stability at high salt concentrations and these extremozymes tolerate high temperature is the industrial application of halophiles [4].

Saline Environment

Microorganisms thrive in high to low range of saline conditions. Extreme conditions come when the concentration of salinity increases, the microorganisms that thrive in these salinity conditions are known as halophiles. They require a saline and alkaline environment to grow [14]. Seawater, hypersaline lakes have salinity imminent to saturation point. These are included in saline environments [15]. The hypersaline environment is found in form of soil and water throughout the world. The domain which possesses high salt concentration than seawater is termed a hypersaline environment [16]. Saline conditions become harsh for microorganisms for their abidance. These conditions become harsh because of some factors depending on the geographical region such as fewer amounts of oxygen, temperature (high or low), and alkalinity, availability of less nutrient, heavy metal, solar radiations, and toxic compounds [17]. Some ions like Na^+ and Cl^- are found in hypersaline medium [18].

The Saline environment includes thalassohaline environment and athalassohaline environment. Thalassohaline environment have its salt ratio similar to that of marine waters. pH of this environment is neutral or slightly alkaline. It has two common kinds of environment; lagoons or coastal hypersaline ponds and marine solar costal. This results in the detection and isolation of halo archaeal species *Haloquadratum walsbyi*. Its strain C23T is the example of thalassohaline environment [1].

Biological components for example; limited rainfall, high temperature, high wind speed, and low humidity causes evaporation in seawater and this result in developing a thalassohaline environment [19, 20]. Na^+ and Cl^- concentrations were found higher in `the environment than in seawater. Some ionic concentration remains similar in seawater and in this environment. Evaporation causes some changes in the ionic composition of gypsum precipitation ($CaSO_4.2H_2O$) and the solubility of minerals. For example; in the Great Salt Lake, Utah, ions such as Na^+ and Cl^- are primary ions in solution. This environment is determined by alkalinity around 7-8 pH [18]. Athalassohaline environment has a difference in ionic composition from seawater [1, 18]. Examples of this environment are the lake of Magadi, carbonate lakes, some alkaline soda lakes, alkaline soil, saltern brines, and the lake of Wadi Narum [20]. Main contents in hyper saline environment are such as; three main cations Na^{2+}, Mg^{2+}, K^+ and anions CO_3^{2-} SO_4^{2-} Cl^-. For example; in Dead Sea concentration of intimidated ions are Mg^{2+} (1.9 M), Na^{2+} (1.6 M) and K^+ (0.14 M) with low pH [61, 18].

Table 1: Comparison in characteristics of Thalassohaline Environment and Athalassohaline Environment [1, 21]

S.No.	Ion (g/l)	Seawater	Thalassohaline Environment (Great Salt Lake)	Athalassohaline Environment (Dead Sea Lake)	Sambhar Lake (Main Lake)
1.	K^+	0.4	7.3	6.7	0.50
2.	Na^+	10.8	39.2	105.4	37.50
3.	Mg^+	1.3	40.7	11.1	0.00
4.	Cl^-	19.6	212.4	181.4	21.46
5.	Br^-	0.1	5.1	0.2	-
6.	Ca^+	0.4	16.9	0.3	0.00
7.	pH	8.2	5.9-6.3	7.7	9.0
8.	Salinity	35.2	322.6	333.6	71.27
9.	SO^{4-}	2.7	0.5	27	6.00
10.	CO_3^{2-}	0.1	0.2	0.7	0.60

Alkaline salt lakes are distributed all over the world in a halassohaline environment in (Table 2). These are formed naturally and present in semi-arid areas. Soda Salt Lake is a naturally occurring lake with high saline conditions and high pH which is required for the development of haloalkane microorganisms.

Climate control, the topography of the area, and geological influences; these are the main cause for the formation of soda Salt Lake [22]. The concentration of sodium carbonate and other complexes such as sodium chloride increases due to the evaporation process that forms the saline-alkaline condition in Soda Lake [23].

The high amount of CO_3^{2+} and Cl^- and low amount of Ca^{2+} and Mg^{2+} ions at a pH range of 8-12 provides a favorable environment to the halophiles [21, 24].

Table 2: Soda Lake distribution all over the world.

Country	Name of Lake	Reference
India	Lake Sambhar and Lake Lonar	25
China	Lake Qinghai, Lake Zhing Hu, Lake Wulanwula Hu, Lake Namu Cuo, Lake Selin Cuo, Lake Lop Nor Manasi Hu, Lake Ulungur Hu and Lake Arakekumu Hu, Lake Mongolian, Lake Aiding Hu, Lake Lop, Lake Chakayan Hu, Lake Terinam, Lake Zarinanmu, Lake	26
Iran	Fars Lake, Oromiea Lake and Persian Gulf.	27
British Columbia	Lake Bowers, Lake Boitano, Lake White, Lake Long, Lake lronmask	28
Kenya	Lake Magadi, Lake Bogoria, Lake Nakuru, Lake Elmenteita, Lake Oloidien, Lake Solai, Lake Sonachi, Lake Sadhana	29
Tanzania	Lake Natron, Lake Eyasi, Lake Magad, Lake Manyara, Lake Balangida, Lake Rukwa, Bosotu Crater Lake, Lake Kusare, Lake Tulusia and Lake Lekandiro	30,31
Uganda	Lake Mahenga, Lake Katwe, Lake Rukwa North, and Lake Kikorongo	32
Saskatchewan, Canada	Lake Quill , Lake Deadmoose, Lake Waldsea, Lake Manitou, Lake Lenore, Lake Wakaw, Lake Frederick, Lake Whiteshore, Lake Muskiki, Lake West Chaplin, Lake Arthur.	33
Egypt	Lake Qarun	34
Russia	Golden Lake, Lake Baskunchak, Elton Lake.	35
Tibet Plateau	Jianshui Lake, Zabuye Salt Lake, Xiangyang Lake, Chaoyang Lake, Yang Lake, Zhenquan Lake, Dujiali Lake, Qingche Lake, Danshui Lake, Wanquan Lake, Luotuo Lake, Deyu Lake	36

Halophiles

The term halophiles were described in 1956. These microorganisms grow at high saline conditions [37]. There are two strategies of halophiles: high-salt-in strategy and low-salt, organic-solutes-in strategy. These strategies insist halophilic and halo-tolerant microorganisms thrive in alkaline and saline conditions. Intracellular proteins should be active and stable in the "High-salt-in" strategy in presence of a molar concentration of KCl and some salts for example; *Halobacteriaceae*, the anaerobic *Halanaerobiales* and *Salinibacter*. The "Low-salt, organic-solutes-in" strategy involves the collection of organic solutes that are not involved in the mechanism of normal enzymes [38]. Halophiles survive in a large group of saline conditions such as saline soils, salted foods, and soda lakes. They are extremophilic micro-organisms [39]. They are of three types based on salt concentration: slight halophiles optimally grow at 0.2-0.5 M NaCl concentration, moderately halophiles optimally grow at 0.5-2.5 M NaCl concentration and extreme halophiles optimally grow at 2.5-5.2 M NaCl concentration [40]. Halophilic microorganisms can maintain osmotic pressure; they are methanogenic, heterotrophic bacteria, heterotrophic eukaryotes, photosynthetic bacteria16. To maintain the osmotic balance in a cell; they use two strategies [41]. The strategy is to maintain its osmotic pressure by accumulating more potassium (K^+) and chloride (Cl^-) ions inside its cell than in its external saline environment. The strategy is also known as the 'salt in strategy. During salt saturation condition this strategy help in maintaining activity and stability of internal proteins [42].

Halophilic microorganisms which survive in high saline conditions, produces an enzyme named halophilic lipases. They have advancement in its industrial application such as in wastewater treatment, food flavor modification, and biodiesel production which provide methodological and theoretical references for research purpose [43]. It was found that halophiles aimed towards the treatment of fish market waste-water and energy production under the saline conditions. Some bacterial strains found during this research was *Bacillus, Ochrobactrum, Rhodococcus, Marinobacter, Microbacterium, Pseudomonas, Martelella, Stenotrophomonas, Xanthobacter* and *Flavobacterium* [44].

Halophiles are divided into three domains such as archaea, bacteria and eukarya. In the domain archaea, the halophilic archaea of class halo bacteria are salt resistant and salt requiring microorganisms. Haloarchaea thrive in high salt environment include salt lakes, salty soils and brines. Red carotenoid pigments found in salt lakes of haloarchaea offers crimson color. Growth of halo bacteria needs from about 12% salt, and for good growth they require 20-25% salt concentration. Moderately halophilic haloarchaea grow optimum around 10% salt concentration. Earlier haloarchaea are not classified on basis of their metabolic properties. They are detected by 16S rRNA sequencing. Examples of haloarchaea include *Haloferax volcanii, Halobacterium spp.* They are facultative anaerobes or both strict aerobes. They are capable to grow on complex media such as peptone and yeast extract. They are highly adapted to high saline conditions. Halobacteria are prokaryotic, they do not have rigid cell wall. They have only single layer made up of glycoprotein known as S-layer. Haloarchaea are lysed due to the threatened nature of their S-layer [45]. In extreme conditions of halophilic microbe, they are classified according to their chemical or physical conditions such as low and high salinity, high and low temperature, acidicity or alkanity, oxygen concentration, pressure, etc. [46, 47]. It was found that the domain of halophiles such as archaea and bacteria has been isolated from the salted skin of sheep. Isolated microbes can cause damage to the collagen fiber of the salted skin of sheep [48].

Classification of halophiles

Halophiles require a saline environment for growth, and they are classified as moderate, extreme, and mild halophiles based on their salt concentration. Moderate halophiles can grow at 7-15% (w/v) of salt concentrations, extreme halophiles can grow more than 15% (w/v) and mild halophiles can grow optimally at 1-6% (w/v) of salt concentrations. For adapting hypersaline environment that is accumulated by K^+ salts or organic solutes are known as compatible solutes. And they maintain osmotic pressure. They are used on an industrial level under its hypersaline environment. They secrete some hydrolytic enzymes such as protease, amylase, xylanase, and cellulose [11]. Recent research found that *Yangia* sp. ND199 is isolated in northern Vietnam which is moderately halophilic bacteria. The bacteria have the tendency to accumulate polyhydroxyalkanoates (PHAs) with the production of exopolysaccharides (EPSs) [49]. *Haloferax volcanii* is the example of extremely halophilic archaeon bacteria which is isolated from Dead Sea which grows with the generation time of 2 h and optimally at 45 °C. This bacterium can tolerate up to 1.8-3.5 M salt concentrations of NaCl [50]. *Salinivibrio* sp. TGB10 is moderately halophilic bacterium which produces polyhydroxyalkanoates (PHB) [51]. *Vibrio alginolyticus* is the example of mild Halophilic bacteria. This bacterium causes wound infection and otitis [52].

Sambhar Lake

Naturally occurring lake with the hypersaline and alkaline environment is situated in the metamorphic rock of Rajasthan (Latitude 26°58'N Longitude 75°05'E). It is also known as Sambhar Soda Lake due to its alkaline conditions [53]. From mean sea level its altitude is 360 meters above [54]. The basin of the Sambhar Lake is covered by Aravalli hills from its north and west direction and separate into two parts; semi-arid part and sub-humid eastern part. The semi-arid part is also known as the Thar Desert [55]. 3 and 1 meters are the maximum and minimum depth of these lakes respectively. In the Sambhar Salt Lake sedimentary deposits are depicted by a bed slit which is formed horizontally [56].

The climatic conditions of Sambhar Lake are tropical. Its climate is based on the seasons such as winter, rain, and summer. In winter's temperature goes below 4 °C and in summer (May to June) the temperature reaches up to 45 °C because the surface of sambhar lake go-through with desiccation which results in the formation of an efflorescent crust but from evidence, no complete desiccation showed [55,56,57]. In the crust, halite and calcite are the main contents. These contents increase the solute (Ca^+, Mg^+, and Na^+) amount in Lake. The rivers such as Roopangarh and Mendha rivers fed the Sambhar Salt Lake during the monsoon season [58]. In the year 1983 to 1985 the annual rainfall range in Sambhar Lake was about 50 cm, from 2005 to 2006 it ranges 39 cm [59, 60]. In Lake 30-40 cm was the average annual rainfall recorded [61]. This lake possesses great geological importance because of its chemical and physical characteristics [62]. Geochemical characteristics such as mineralogical studies, water evaporation from brine by using oxygen isotope, the hydrological system in sambhar Salt Lake water, monsoon, and climate variability [55,57,63,64]. The lake is in shallow shape with a length of 22.5 km; width goes up to 11.2 km and covers approx. 225 sq. Km [65]. Water depends on the two main streams of water that conjugate; Mendha and Rupangarh. The river Mendha flows from the northeast and followed southwest direction. The river Rupangarh flows from Ajmer city and enters into the lake of the southern hilly area [54,63].

Diversity of halophiles in Sambhar Lake

Three domains of halophiles such as archaea, bacteria, eukarya tend to grow in hypersaline and alkaline soda salt lakes. An environmental physical characteristic plays a major role in the habitat of microbial diversity [66]. Soda salt lakes are found in countries such as Africa, India, etc. and they all possess the same characteristics of saline environment such as alkalinity and salinity [18]. Some studies emphasize haloalkaliphiles and halophiles for their phylogenetic diversity in terms of their saline and alkaline conditions. Researchers isolated six variants of haloalkaliphiles from Sambhar Lake while studying their chemical composition in 1990 and 1994. These strains possess features like archaeal bacteria in terms of their lipid composition that is analyzed by carotenoid and lipid extraction and are assigned as a new genus, *Natronobacterium*. These variants were cultured at pH 9.0 with 15% salinity [10,64]. Some species have been reported from bacillus group such as *Bacillus subtilis, B.amyloliquefaciens, B.sphaericus, B.licheniformis*) and some species of halobacterium *(Staphylococcus capitis and Micrococcus*) have been reported [60]. *Halobacterium* with 20% salinity produces an orange color bacterial pigment called rhodopsin. The researcher work on the growth of halophilic phototrophic anoxygenic sulfur-oxidizing bacteria belongs to the genus *Ectothiorhodospira* [53]. The genomic DNA of microorganisms that are isolated from the soil of Sambhar Lake is extracted with help of molecular techniques or metagenomics68. From Sambhar Lake, a bacteria named *Gracilibacillus* sp. TSCPVG was isolated. This bacterium is an aerobic halophilic bacterium that produces an enzyme named xylanase. This enzyme grows in a thermostable environment at a pH of 6.5-10.5 with a 1-30% salinity range [69]. Based on 16S rRNA gene sequencing and biochemical test, 38 halophilic variants of genus *Staphylococcus*, *Geomicrobium*, and *Bulleidia* are found. *Geomicrobium* sp. EMB2 can produce protease enzymes that provide stability to survive at higher pH (7.0-12.0) [70]. *Halomonas* sp. and *Nitrinicola lacisaponesis* are the two variants with 0-22% salinity tolerant and 0-12% salinity tolerant respectively [71]. From Sambhar Lake, ninety-three bacteria which are haloalkaliphilic in nature are isolated. They grow with a pH of 6-12 and 25% salinity. These isolated bacteria are then divided into 32 groups based on 16S rRNA sequencing. 53.2% of bacteria have shown some similarity with the phylum *Firmicutes*, 40.63% similarity with the *Proteobacteria,* and the remaining 6.25% similarity with *Actinobacteria.* 108 halophilic bacteria were examined by researchers for the manufacture of industrially valuable enzymes for example; amylases, lipases, and proteases [72]. In saline stress conditions halophilic bacteria help in plant growth. Production of indole-3-acetic acid (IAA) showed positive and manufacturing of siderophore. These both help in the promotion of growth [73]. In the detergent industry, alkaline proteases play a major role in the removal of proteinaceous strain. *Bacillus subtilis*, an alkaline protease enzyme found stable at 87-105% of its residual activity, when it gets compared with the detergents found in the market at the commercial level [74]. From Sambhar Lake, halophilic bacterial variant *Bacillus* sp. performed a keratinolytic activity by using feathers of chicken [75]. A solid form of hexavalent chromium Cr (VI) is mutagenic, toxic, and causes harm to the human body which is used at an industrial level. In salt-loaded wastewater a halotolerant bacterium named *Halomonas* sp. CSB 5 acts as a reducer or a bioremediating agent [76]. In the early stages of growth of the haloalkaliphilic bacteria named *Halobiforma* sp. variant BNIITR, it is rod shape gram-negative and in its stationary or resting phase, it is coccoid shaped gram-negative cells. This strain produces protease enzyme and for its growth, it requires a pH of 8-8.5 with 18% salinity [77]. In sambhar lake halophilic bacteria diversity includes Bacillus such as; *Bacillus decolorationis, B. halodurans, B. safensis, Halobacillus dabanensis, B. vallismortis, B. methanolicus, Oceanobacillus manasiensi* and *H. Trueperi* [78]. Moderately halophilic bacteria isolated from the diversity of halophilic bacteria based on biochemical, physiological, and morphological characteristics [79]. The bacterium that helps plants to grow under stress conditions is *Pseudomonas aeruginosa* KP163922 [80]. The variant Bacillus *licheniformis* HSW-16 produces the enzyme protease and was detected and confirmed by amplification. This variant grows with 9% salinity and a pH of 7 -10 [81]. From different areas of Sambhar Lake, the diversity of microorganisms such as haloalkaliphilic gammaproteobacteria found that the nine isolated bacteria belong from one genus *Halomonas*. This genus *Salomon* has four species as *Halomonas venusta, Halomonas alkaliphilic, Halomonas pantelleriensis, and Halomonas* sp. These isolated bacterial species grow at a pH of 8-12 and with 10-20% salinity [82]. In Sambhar Lake moderately halophilic bacterium such as

Table 3: Diversity of halophilic bacteria in the Sambhar Lake with their applications.

S. No.	Bacteria	Applications	References
1.	*Dunaliella parva*	This alga used as a source of food in aquaculture. It assembles nutrients such as vitamins, proteins and lipids.	84
2.	*Halomonas shambharensis*	Used in the osmoprotectant strategies to overcome salinity stress. Used in investigation of protein coding gene.	85
3.	*Dunaliella salina*	Used in biosorption of Cr (VI) Bioremediation of hexavalent Cr (VI) in presence of aqueous solution.	86
4.	*Natrialba swarupiae* sp. nov.	Their Cells are coccoid, non-motile, lyse in distilled water, Gram-stain-negative and 0–5% NaCl	87
5.	*Salisediminibacterium halotolerans*	Used in bioethanol production. It is endoglucanase halo stable bacteria	88
6.	*Thalassobacillus*	Application includes, biofuels production bioactive compounds, bioremediation, biosurfactants and plant growth promotion.	89
7.	*Paenibacilllus* sp. SMB1	Used for characterization of antimicrobial compound with the help of amino acid analysis and mass spectroscopy	90
8.	*Piscibacillus* sp.	Show anticancer action against breast cancer. It can hydrolyze skimmed milk.	91
9.	*Anabaenopsis* sp. SLCyA	UV absorbing compounds are present. They produce large quantity of MAA molecules. These MAA provide protection to E. coli against UV- B radiation.	92
10.	*Pseudomonas aeruginosa gi*|KP 16392|	Bioremediation and Biosurfactant production are the application	93
11.	*Halomonas* sp. Strain SBS 10	Its isolation gives deep understanding about gene clusters and its adaptation to saline environment.	94
12.	*Lentibacillus* sp. NS12IITR	Production of lipids to improve biodiesel properties with the production of hydrocarbon.	95

Conclusion

Halophilic microorganisms live in extreme saline environment such as thalassohaline environment and athalassohaline environment. They can cope up with osmotic stress by accumulating more chloride and potassium ions. Some of the main application of halophiles is that it is used in bioremediation, medicine and agricultural purpose. In Sambhar Lake huge diversity of halophilic bacteria are found that includes mild, moderate and extreme halophiles. The chemical and physical characteristics provide great geological importance to the Sambhar Lake.

Conflict of interest

All listed authors are declared that they have no conflict of interest.

Acknowledgements

All listed authors are thankful to Department of Microbiology, JECRC University for providing the related support to compile this work.

References

1. Cui, H. L., & Dyall-Smith, M. L. Cultivation of halophilic archaea (class Halobacteria) from thalassohaline and athalassohaline environments. *Marine Life Science & Technology* 2021; 3(2), 243-251.
2. Rodriguez-Valera, F. Introduction to saline environments. In *The biology of halophilic bacteria* 2020; 1-23,CRC Press.
3. DasSarma, S., & Arora, P. Halophiles. Encyclopedia of life sciences. *Nature Publishishing* Group 2001; 1-9.
4. De Lourdes Moreno, M., Pérez, D., García, M. T., & Mellado, E. Halophilic bacteria as a source of novel hydrolytic enzymes. *Life* 2013; 3(1), 38-51.
5. DasSarma, S. Halophiles, industrial applications. *Encyclopedia of industrial biotechnology: bioprocess, bioseparation, and cell technology* 2009;1-43.
6. Dumorné, K. Biotechnological and industrial applications of enzymes produced by extremophilic bacteria. A mini review. *Life Sciences* 2018; 1-22.
7. Roberts, M. F. Organic compatible solutes of halotolerant and halophilic microorganisms. *Saline systems* 2005; *1*(1), 1-30.
8. Ali-Begloui, M., Salehghamari, E., Sadrai, S., Ebrahimi, M., Amoozegar, M. A., & Salehi-Najafabadi, A. Biotransformation of trinitrotoluene (TNT) by newly isolated slight halophilic bacteria. *Microbiology* 2020; 89(5), 616-625.
9. Corral, P., Amoozegar, M. A., & Ventosa, A. Halophiles and their biomolecules: Recent advances and future applications in biomedicine. Marine drugs 2019; 18(1), 1-33.
10. Upasani, V., and Desai, S. Sambhar salt lake. *Archives of microbiology* 1990; 154(6), 589-593.
11. Mitra, R., Xu, T., Xiang, H., & Han, J. Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. *Microbial cell factories* 2020*;* 19(1), 1-30.
12. Li, X., Gan, L., Hu, M., Wang, S., Tian, Y., & Shi, B. *Halomonas pellis* sp. nov., a moderately halophilic bacterium isolated from wetsalted hides. *International Journal of Systematic and Evolutionary Microbiology* 2020; 70(10), 5417-5424.
13. Oyewusi, H. A., Wahab, R. A., & Huyop, F. Dehalogenase-producing halophiles and their potential role in bioremediation. *Marine Pollution Bulletin* 2020*;*160, 111603.
14. Antón, J., Rosselló-Mora, R., Rodríguez-Valera, F., & Amann, R. Extremely halophilic bacteria in crystallizer ponds from solar salterns. *Applied and environmental microbiology* 2000; 66(7), 3052-3057.
15. Gunde-Cimerman, N., Plemenitaš, A., & Oren, A. Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations. *FEMS microbiology reviews* 2018; 42(3), 353-375.
16. Oren, A. Halophilic microbial communities and their environments. *Current opinion in biotechnology* 2015; 33**,** 119-124.
17. Rodriguez-Valera, F. Characteristics and microbial ecology of hypersaline environments. *Halophilic bacteria* 1988; 1**,** 3 -30.

18. Oren, A. (2002). Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology and Biotechnology* 2002;28(1), 56-63.
19. McGenity, T.J., Gemmell, R.T., Grant, W.D., and Stan-Lotter, H. Origins of halophilic microorganisms in ancient salt deposits. *Environmental Microbiology* 2000; 2(3), 243-250.
20. Safarpour, A., Amoozegar, M.A., and Ventosa, A. "Hypersaline Environments of Iran: Prokaryotic Biodiversity and Their Potentials in Microbial Biotechnology," in *Extremophiles in Eurasian Ecosystems: Ecology, Diversity, and Applications*. Springer 2018;8, 265-298.
21. Jones, B. E., Grant, W. D., Duckworth, A. W., & Owenson, G. G. Microbial diversity of soda lakes. *Extremophiles* 1998; 2 (3), 191-200.
22. Tindall, B.J. Prokaryotic life in the alkaline, saline, athalassic environment. *Halophilic bacteria* 1988; 1, 31-67.
23. Sorokin, D.Y., Berben, T., Melton, E.D., Overmars, L., Vavourakis, C.D., and Muyzer, G. (2014). Microbial diversity and biogeochemical cycling in soda lakes. *Extremophiles* 2014; 18(5), 791-809.
24. Lee, C. J., McMullan, P. E., O'Kane, C. J., Stevenson, A., Santos, I. C., Roy, C., & Hallsworth, J. E. NaCl-saturated brines are thermodynamically moderate, rather than extreme, microbial habitats. *FEMS microbiology reviews* 2018; *42*(5), 672-693.
25. Singh, D., Singh, P., Asthana, H., Roy, N., & Mukherjee, S. Contamination of water resources in and around saline lakes. *Contamination of Water* 2021;19-29.
26. Williams, W. Chinese and Mongolian saline lakes: a limnological overview. *Hydrobiologia* 1991; 210(1-2), 39-66.
27. Hasan, S. M., & Mohammadian, J. Isolation and characterization of *Halobacterium salinarum* from saline lakes in Iran. *Jundishapur Journal of Microbiology* 2011; 4(1), 59-65.
28. Scudder, G. G. E. The fauna of saline lakes on the Fraser Plateau in British Columbia: With 7 figures and 4 tables in the text. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen* 1969;17(1), 430-439.
29. Mwaniki, P., Taita, T., Sierens, T., & Triest, L. Barriers to genetic connectivity of smooth flatsedge (Cyperus laevigatus) among alkaline-saline lakes of Eastern Rift Valley (Kenya). *Aquatic Botany* 2019;155, 38-44.
30. Grant, W., and Jones, B. Alkaline environments. *Encyclopaedia of microbiology* 2000;1, 126-133.
31. Scoon, R.N. "Lakes of the Gregory Rift Valley: Baringo, Bogoria, Nakuru, Elmenteita, Magadi, Manyara and Eyasi," in *Geology of National Parks of Central/Southern Kenya and Northern Tanzania*. Springer 2018; 167-180.
32. Grant, W.D., and Sorokin, D.Y. "Distribution and diversity of soda lake alkaliphiles," in *Extremophiles handbook*. Springer 2011; 1, 27-54.
33. Hammer, U. T., Shamess, J., & Haynes, R. C. The distribution and abundance of algae in saline lakes of Saskatchewan, Canada. *Hydrobiologia* 1983;105(1), 1-26.
34. Mohamed, A. S., Gad, N. S., & El Desoky, M. A. Liver Enzyme Activity of Tilapia zillii and Mugil capito Collected Seasonally from Qarun Lake, Egypt. *Fisheries and Aquaculture Journal* 2019;10(1), 1-5.
35. Brylev, V. Russian saline lakes Elton and Baskunchak as challengers to the UNESCO World Heritage List. *Folia Geographica* 2019; 61(1), 87.
36. Yan, L., & Zheng, M. Influence of climate change on saline lakes of the Tibet Plateau, 1973–2010. *Geomorphology* 2019; 246, 68-78.
37. Flannery, W.L. Current status of knowledge of halophilic bacteria. *Bacteriological reviews* 1956; 20(2), 49.
38. Ma, Y., Galinski, E. A., Grant, W. D., Oren, A., & Ventosa, A. Halophiles 2010: life in saline environments. *Applied and environmental microbiology*, 2010; 76(21), 6971-6981.
39. Enache, M., Teodosiu, G., Itoh, T., Kamekura, M., and Stan-Lotter, H. "Halophilic microorganisms from man-made and natural hypersaline environments: Physiology, ecology, and biotechnological potential," in *Adaption of Microbial Life to Environmental Extremes*. Springer 2017; 201-226.
40. Samad, N.S.A. Isolation and identification of halophilic bacteria producing halotolerant protease. *Science Heritage Journal,* 2017;1(1), 07-09.

41. Weinisch, L., Kühner, S., Roth, R., Grimm, M., Roth, T., Netz, D.J., et al. Identification of osmoadaptive strategies in the halophile, heterotrophic ciliate *Schmidingerothrix salinarum*. *PLoS biology* 2018;16(1), 1-29.
42. Banciu, H.L., and Muntyan, M.S. Adaptive strategies in the double-extremophilic prokaryotes inhabiting soda lakes. *Current opinion in microbiology* 2015; 25, 73-79.
43. Qiu, J., Han, R., & Wang, C. Microbial halophilic lipases: A review. *Journal of Basic Microbiology* 2021; 61(7), 594-602.
44. Jamal, M. T., & Pugazhendi, A. Treatment of fish market wastewater and energy production using halophiles in air cathode microbial fuel cell. *Journal of Environmental Management* 2021;292, 112752.
45. Smith, M. D. Protocols for haloarchaeal genetics. *The halo handbook version* 2008; 6, 11-15.
46. Prieur, D. & Marteinsson, V. T. Prokaryotes living under elevated hydrostatic pressure. *In Biotechnology of Extremophiles* 1998; 61, 23–35.
47. Oarga, A. (2009). Life in extreme environments. *Revista de Biologia e ciencias da Terra 9*(1), 1-10.
48. Enquahone, S., van Marle, G., Gessesse, A., & Simachew, A. Molecular identification and evaluation of the impact of red heat damage causing halophilic microbes on salted hide and skin. *International Biodeterioration & Biodegradation* 2020;150, 1-10.
49. Romero Soto, L., Thabet, H., Maghembe, R., Gameiro, D., Van-Thuoc, D., Dishisha, T., & Hatti-Kaul, R. Metabolic potential of the moderate halophile *Yangia* sp. ND199 for co-production of polyhydroxyalkanoates and exopolysaccharides. *Microbiologyopen* 2021; 10(1), 1-13.
50. Haque, R. U., Paradisi, F., & Allers, T. *Haloferax volcanii* for biotechnology applications: challenges, current state and perspectives. *Applied microbiology and biotechnology* 2020;104(4), 1371-1382.
51. Tao, G. B., Tan, B. W., & Li, Z. J. (2021). Production of polyhydroxyalkanoates by a moderately halophilic bacterium of *Salinivibrio* sp. TGB10. *International Journal of Biological Macromolecules* 2021;186, 574-579.
52. Huang, L., Guo, L., Xu, X., Qin, Y., Zhao, L., Su, Y., & Yan, Q. The role of rpoS in the regulation of *Vibrio alginolyticus* virulence and the response to diverse stresses. *Journal of fish diseases* 2019; 42(5), 703-712.
53. Upasani, V.N. "Microbiological Studies on Sambhar Lake (Salt of Earth) Rajasthan, India", in: *Proceedings of Taal2007: The 12th World Lake Conference*) 2008; 448-450.
54. Yadav, D., Sarin, M., and Krishnaswami, S. (2007). Hydrogeochemistry of Sambhar Salt Lake, Rajasthan: implication to recycling of salt and annual salt budget. *Journal Geological Society of India* 2007; 69(1), 139.
55. Sinha, R., and Raymahashay, B. Evaporite mineralogy and geochemical evolution of the Sambhar Salt Lake, Rajasthan, India. *Sedimentary Geology* 2004;166(1-2), 59-71.
56. Deshmukh, S. K., Verekar, S. A., & Chavan, Y. G. Keratinophilic fungi from the vicinity of salt pan soils of Sambhar lake Rajasthan (India). *Journal de Mycologie Médicale* 2018; 28(3), 457-461.
57. Sinha, R., Smykatz-Kloss, W., Stüben, D., Harrison, S., Berner, Z., and Kramar, U. Late Quaternary palaeoclimatic reconstruction from the lacustrine sediments of the Sambhar playa core, Thar Desert margin, India. *Palaeogeography, Palaeoclimatology, Palaeoecology* 2006; 233(3-4), 252-270.
58. Yadav, D., and Sarin, M. Geo-chemical Behavior of Uranium in the Sambhar Salt Lake, Rajasthan (India): Implications to" Source" of Salt and Uranium" Sink". *Aquatic Geochemistry* 2009a; 15(4), 529.
59. Yadav, D. Oxygen isotope study of evaporating brines in Sambhar Lake, Rajasthan (India). *Chemical Geology* 1997; 138(1-2), 109-118.
60. Sundaresan, S., Ponnuchamy, K., and Rahaman, A.A. "Biological management of Sambhar lake saltworks (Rajasthan, India)", in: *Proceedings of the 1st International Conference on the Ecological Importance of Solar Saltworks* 2006; 199-208.
61. Binayke, A., Ghorbel, S., Hmidet, N., Raut, A., Gunjal, A., Uzgare, A., & Nawani, N. Analysis of diversity of actinomycetes from arid and saline soils at Rajasthan, India. *Environmental Sustainability* 2018; 1(1), 61-70.
62. Sinha, R., and Raymahashay, B. (2000). Salinity model inferred from two shallow cores at Sambhar salt lake, Rajasthan. *Journal Geological Society of India* 2000;56(2), 213-218.
63. Yadav, D., and Sarin, M. Ra–Po–Pb isotope systematics in waters of Sambhar Salt Lake, Rajasthan (India): geochemical characterization and particulate reactivity. *Journal of environmental radioactivity* 2009b; 100(1), 17-22.
64. Kumar, O., Devrani, R., and Ramanathan, A. Deciphering the Past Climate and Monsoon Variability from Lake Sediment Archives of India: A Review. *Journal of Climate Change* 2017;3(2), 11-23.

65. Pathak, A.P., and Cherekar, M.N. Hydrobiology of hypersaline Sambhar salt Lake a Ramsar site, Rajasthan, India. *Indian Journal of Geo-Marine Sciences* 2015; 44, 1640–1645.
66. DasSarma, S., and DasSarma, P. Halophiles. *eLS* 2012;1-9.
67. Upasani, V.N., Desai, S.G., Moldoveanu, N., and Kates, M. Lipids of extremely halophilic archaeobacteria from saline environments in India: a novel glycolipid in *Natronobacterium* strains. *Microbiology* 1994; 140(8), 1959-1966.
68. Siddhapura, P., Vanparia, S., Purohit, M., and Singh, S. Comparative studies on the extraction of metagenomic DNA from the saline habitats of Coastal Gujarat and Sambhar Lake, Rajasthan (India) in prospect of molecular diversity and search for novel biocatalysts. *International journal of biological macromolecules* 2010; 47(3), 375-379.
69. Giridhar, P.V., and Chandra, T. Production of novel halo-alkali-thermo-stable xylanase by a newly isolated moderately halophilic and alkali-tolerant *Gracilibacillus* sp. TSCPVG. *Process Biochemistry* 2010; 45(10), 1730-1737.
70. Karan, R., and Khare, S. Purification and characterization of a solvent-stable protease from *Geomicrobium* sp. EMB2. *Environmental technology* 2010; 31(10), 1061-1072.
71. Tiwari, S., Singh, P., Tiwari, R., Meena, K.K., Yandigeri, M., Singh, D.P., et al. Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*Triticum aestivum*) and chemical diversity in rhizosphere enhance plant growth. *Biology and Fertility of soils* 2011; 47(8), 907.
72. Kumar, S., Karan, R., Kapoor, S., Singh, S., and Khare, S. Screening and isolation of halophilic bacteria producing industrially important enzymes. *Brazilian Journal of Microbiology* 2012; 43(4), 1595-1603.
73. Ramadoss, D., Lakkineni, V. K., Bose, P., Ali, S., & Annapurna, K. Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. *SpringerPlus* 2013; 2(1), 1-7.
74. Singhal, P., Nigam, V., and Vidyarthi, A. Studies on production, characterization and applications of microbial alkaline proteases. *International Journal of Advanced Biotechnology and Research* 2012; 3(3), 653-669.
75. Nigam, V., Singhal, P., Vidyarthi, A., Mohan, M., and Ghosh, P. Studies on keratinolytic activity of alkaline proteases from halophilic bacteria. *International Journal of Pharmacy and Biological Sciences* 2013a; 4, 389-399.
76. Chandra, P., and Singh, D. Removal of Cr (VI) by a halotolerant bacterium *Halomonas s*p. CSB 5 isolated from sāmbhar salt lake Rajasthan (India*). Cellular and Molecular Biology* 2014; 60(5), 64-72.
77. Gupta, M., Aggarwal, S., Navani, N.K., and Choudhury, B. (2015). Isolation and characterization of a protease-producing novel haloalkaliphilic bacterium *Halobiforma* sp. strain BNMIITR from Sambhar lake in Rajasthan, India. *Annals of microbiology* 2015;65(2), 677-686.
78. Yadav, A.N., Verma, P., Kumar, M., Pal, K.K., Dey, R., Gupta, A., et al. Diversity and phylogenetic profiling of niche-specific Bacilli from extreme environments of India. *Annals of microbiology* 2015; 65(2), 611-629.
79. Gaur, A., and Mohan, D. Diversity of Moderate Halophilic bacteria from Sambahr Salt Lake. *International journal of current microbiology and applied sciences* 2014; 7, 518-525.
80. Mishra, S., Raghuvanshi, S., & Gupta, S. Deducing the bio-perspective capabilities of Fe (II) oxidizing bacterium isolated from extreme environment. *Biochemistry and Analytical Biochemistry*, 2015; 4(2), 1.
81. Singh, R.P., and Jha, P.N. Characterization and Optimization of Alkaline Protease Production from *Bacillus licheniformis* HSW-16 Isolated from Sambhar Salt Lake. *International Journal of Applied Sciences and Biotechnology* 2015; 3(2), 347-351.
82. Cherekar, M.N., and Pathak, A.P. Studies on Haloalkaliphilic gammaproteobacteria from hypersaline Sambhar Lake, Rajasthan, India. *Indian Journal of Geo-Marine Sciences* 2015;44, 1646-1653.
83. Gaur, A., Prasad, A., and Parveen, A. *Oceanobacillus* Sp. Moderate Halophilic Bacterium Isolated From Sambhar Salt Lake. *International Journal of Pharmacology and Biological Sciences* 2017a; 11(2), 13.
84. Bhargava, A., & Pareek, A. Optimization of Growth Medium and Salt Concentration for the Growth of *Dunaliella parva* isolated from Sambhar Lake (Rajasthan, India). *Journal of Plant Science Research* 2019; 35(2), 229-232.
85. Jadhav, K., Kushwaha, B., Jadhav, I., Shankar, P., Geethadevi, A., Kumar, G., ... & Parashar, D. Genomic analysis of a novel species *Halomonas shambharensis* isolated from hypersaline lake in Northwest India. *Molecular Biology Reports* 2021;48(2), 1045-1053.
86. Kaushik, G., & Raza, K. Potential of novel *Dunaliella salina* from sambhar salt lake, India, for bioremediation of hexavalent chromium from aqueous effluents: An optimized green approach. *Ecotoxicology and environmental safety* 2019;180, 430-438.

87. Kajale, S., Deshpande, N., Pali, S., Shouche, Y., & Sharma, A. (2020). *Natrialba swarupiae* sp. nov., a halophilic archaeon isolated from a hypersaline lake in India. *International journal of systematic and evolutionary microbiology* 2020;70 (3), 1876-1881.

88. Sar, A., Pal, S., Islam, S., Mukherjee, P., & Dam, B. An Alkali-Halostable Endoglucanase Produced Constitutively by a Bacterium Isolated from Sambhar Lake in India with Biotechnological Potential. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 2021;91(2), 319-326.

89. Tuesta-Popolizio, D. A., Velázquez-Fernández, J. B., Rodriguez-Campos, J., & Contreras-Ramos, S. M. *Thalassobacillus,* a genus of extreme to moderate environmental halophiles with biotechnological potential. *World Journal of Microbiology and Biotechnology* 2021; 37(9), 1-13.

90. Singh, H., Kaur, M., Jangra, M., Mishra, S., Nandanwar, H., & Pinnaka, A. K. Antimicrobial properties of the novel bacterial isolate *Paenibacilllus* sp. SMB1 from a halo-alkaline lake in India. *Scientific reports* 2019;9(1), 1-12.

91. Neelam, D. K., Agrawal, A., Tomer, A. K., Bandyopadhayaya, S., Sharma, A., Jagannadham, M. V., ... & Dadheech, P. K. A *Piscibacillus* sp. isolated from a soda lake exhibits anticancer activity against breast cancer MDA-MB-231 cells. *Microorganisms* 2019; 7(2), 34.

92. Bairwa, H. K., Prajapat, G., Jain, S., Khan, I. A., Ledwani, L., Yadav, P., & Agrawal, A. Evaluation of UV-B protection efficiency of mycosporine like amino acid extracted from the cyanobacteria *Anabaenopsis* sp. SLCyA isolated from a hypersaline lake. *Bioresource Technology Reports*, 2021; 15, 1-8.

93. Gaur, S., Gupta, S., & Jain, A. Characterization and oil recovery application of biosurfactant produced during bioremediation of waste engine oil by strain *Pseudomonas aeruginosa gi* |KP 16392| isolated from Sambhar salt lake. *Bioremediation Journal* 2021; 25(4), 308-325.

94. Kushwaha, B., Sharma, G. P., Sharma, A., Shankar, P., Geethadevi, A., Sharma, N., & Jadhav, K. Whole-genome shotgun sequence of *Halomonas* sp. strain SBS 10, isolated from a hypersaline lake in India. *Microbiology resource announcements*, 2020; 9(1), 1-2.

95. Singh, N., & Choudhury, B. Potential of *Lentibacillus* sp. NS12IITR for production of lipids with enriched branched-chain fatty acids for improving biodiesel properties along with hydrocarbon co-production. *Extremophiles* 2018; 22 (6), 865-875.

Citation: Chakravati N, Gupta V, Devki, Rahi RK, Neelam DK. “Halophilic Bacterial Diversity of Sambhar Salt Lake, Rajasthan, India”. *SVOA Microbiology 2022,* 3:4, 59-69.

Copyright: © 2022 All rights reserved by Neelam DK., et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.