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(Review Article)

Halophilic microbiome: Distribution, diversity and applications

Mukundraj Govindrao Rathod [1], Gautam Tanaji Kamble [2], Pratibha Ishwardas Dhawale [2], Tanya Keshavrao Kendre [2], Shalini Ananda Kadam [2], Jivan Munja Dhotare [3], Ramdas Ganpatrao Bhong [2] and Anupama Prabhakarrao Pathak [2, *]

[1] *Department of Biotechnology & Bioinformatics (U.G. & P.G.), Yeshwant College of Information Technology, Parbhani 431401(affiliated to Swami Ramanand Teerth Marathwada University, Nanded), Maharashtra, India.*
[2] *School of Life Sciences (DST-FIST phase-I and UGC-SAP DRS-II sponsored school), Swami Ramanand Teerth Marathwada University, Nanded-431606, Maharashtra, India.*
[3] *College of Agricultural Biotechnology (affiliated to Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani), Hatta 431705, Taluka Vasmat, Dist. Hingoli, Maharashtra state, India.*

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Abstract

Microorganisms that love salt are known as halophiles, and they are found in both the archaeal and eubacterial domains of life. Halophiles do naturally occur in mesmeric saline habitats such as hypersaline lakes, salt pans, salt marshes, saline soils, and solar salterns. The group of microorganisms that live in a salty environment is known as the halophilic microbiome. This group includes bacteria, algae, fungi, viruses, and more. According to how much salt they can tolerate, halophiles are divided into three categories: mild (0.3-0.8 M), moderate (0.8-3.4 M), and extreme (3.4-5.1 M). Seawater already covers the majority of the surface of the Earth and has a salinity of roughly 0.6 M. The most numerous and adaptable microorganisms are therefore those that thrive in such environments. Due to the presence of carotenoids, many halophiles exhibit red-pigmented colonies. This pigment resembles that of tomatoes, red peppers, pink flamingos, and other similar foods. In the production of traditional fermented foods like sauces and pickles, some halophiles play a specific role. Since the majority of their potential is still unknown, halophilic microbes have fewer but more significant biotechnological applications than thermophiles and alkaliphiles. Nevertheless, they could be an important source of many particular biomolecules, including salt-stable enzymes, biopolymers, and pigments. Halophiles may also be crucial for bioprocesses like bioleaching, bioremediation, biotransformation, and biofermentation. Halophiles might have some interesting uses in both medicine and agriculture. This review essay examined the diversity, distribution, and uses of halophiles.

Keywords: Archaea; Habitat; Halophiles; Microbiome; Saline soil; Sodic soil

1. Introduction

One category of extremophiles is the halophiles. Microorganisms that love salt are called halophiles. They are the most extraordinary type of microorganism due to their ability to survive at concentrations of 10–30% NaCl. Eukaryotes, archaea, and bacteria all contain halophiles [1]. Halophiles can be facultatively anaerobic, obligately anaerobic, aerobic, or microaerophilic [2]. According to estimates, saline and sodic soils cover 932 million hectares of the Earth's surface [3]. The majority of the Earth is covered by sea water, and the average ocean salinity is between 33 and 37 g per litre [4]. The oceans contain roughly 96.5 percent of the planet's total water, covering about 71 percent of its surface [5]. Therefore, the most numerous and adaptable microorganisms are those that thrive in these environments and love and tolerate salt. Halophiles have adapted to a few unique and unusual strategies. Halophiles produce compatible solutes in

* Corresponding author: Anupama Prabhakarrao Pathak; Email: anupama.micro@rediffmail.com

their cells or have the transporters necessary to survive in extremely salinity-rich environments. They generate a number of distinct osmo-regulatory solutes, including potassium ions, glutamate, proline, ectoine, and betaine. Some halophiles produce acidic proteins that can work in environments with a lot of salt [6]. Table 1 shows a few halophiles along with the source of their isolation.

Table 1 Some halophilic microorganisms and their habitats

Halophiles	Source of isolation	Reference
Halobacillus andaensi *Halobacillus dabanensis* *Marinococcus luteus* *Alkalibacillus salilacus* *Bacillus halochares* *Aquisalibacillus elongatus*	Saline-sodic soil of Cuatro Cienegas Basin located in the north of Mexico in Coahuila state (27º 00' N and 101º48' 49'' W)	[3]
Halobacillusdabanensis *Marinococcus luteus* *Alkalibacillus filiformis*	Soils from Sayula lake Mexican geographical areas	[3]
Halobacillus dabanensis *Marinococcus luteus* *Alkalibacillus filiformis*	Soils from San Marcos lake, Mexican geographical areas	[3]
Halobacillus sp.	Salt marsh, saline soils and fermented foods	[3]
Marinococcus sp.	Seawater, solar salterns, and saline soils	[3]
Nesterenkonia sp. *Micrococcus* sp. *Kocuria* sp. *Salinicoccus* sp. *Gracibacillus* sp. *Bacillus* sp.	Texcoco lake	[3]
Idiomarina sp. *Salicola sp.* *Halomonas sp.* *Pseudomonas sp.* *Marinobacter sp.*	Urmia Lake, Iran (second largest salt water lake on the Earth)	[7]
Vibrio sp. *Pseudoalteromonas sp.* *Halomonas sp.,* *Marinobacter sp.,* *Idiomarina sp.*	Saltern ponds in Taean Gun, Korea	[7]
Halococcus sp.	Seawater sample	[8]
Staphylococcus sp. *Stenotrophomonas sp.* *Bacillus sp.* *Pseudomonas sp.* *Enterobacter sp.* *Ochrabactrum sp.* *Oceanobacillus sp.*	Indian salt pans- Thoothukudi (8°47′N, 78°8′E), Kanyakumari (8°08′N, 77,054′E), Marakkanam (12,018′N, 79,092′E), Ernakulam (9°9′N, 76°2′E) and Ribandar (15°30′N, 73°51′E)	[9]

Halorubrum, *Haloarcula,* *Halomonas,* *Halovibrio,* *Salicola,* *Salinibacter genera*	Exportadora de Sal saltworks in Guerrero Negro, Baja California, Mexico	[10]
Haloalkaliphilic bacterium EMB1 *Haloalkaliphilic bacterium EMB2* *Haloalkaliphilic bacterium EMB3* *Haloalkaliphilic bacterium EMB4*	Soil and water samples were collected from Sambhar Salt Lake (Rajasthan, 26°58′N75°05′E), India	[11]
Marinobacter sp. EMB5 *Marinobacter sp. EMB6* *Virgibacillus sp. EMB7* *Marinobacter sp. EMB8*	sea coast of Kozhikode (Kerala, 11°25′N75°77′E), India	[11]
Bacillus sp. EMB9 *Oceanobacillus sp. EMB10*	Goa (Goa, 15°59′N73°73′E , India	[11]
Chromohalobacter sp. EMB12 *Virgibacillus sp. EMB13* *Halobacillus sp. EMB14*	Triveni Sangam (Gujarat, 20°71′N70°97′E), India	[11]
Halobacillus sp. EMB15 *Virgibacillus sp. EMB16* *Halobacillus sp. EMB1* *Haloalkaliphilic bacterium D-10-102*	Nagoa (Diu, 20°71′N70°92′E), India	[11]
Halomonas sp. EMB11 *Haloalkaliphilic bacterium EMB18* *Haloalkaliphilic bacterium S-15-9*	Somnath (Gujarat, 20°88′N70°40′E), India	[11]
Haloalkaliphilic bacterium Ve2-20-92	Veraval (Gujarat, 20°91′N70°35′E), India	[11]
Haloferax volcani *Halobacterium sodomense*	Dead sea coast, Jordan	[12]
Members of the genera: *Salicola* *Bacillus* *Halorubrum* *Natrinema* *Haloterrigena*	Chott El Jerid, a hypersaline lake in the south of Tunisia	[13]
Halorubrum sp.	Solar saltern of Mulund, Mumbai, India	[14]

Many researchers have found various groups of halophiles, alkaliphiles, and haloalkaliphiles from saline-alkaline soil and water samples and identified them by polyphasic approach. They have also reported their bio-industrial importance. Many such findings are in addition to the genera and species mentioned above. [15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32]. The majority of the Earth's surface is covered by seas and oceans, which make up about 71% of its total surface area. In these marine environments with water that contains 3% salt, the majority of halophiles thrive. Brine is created when the dissolved mineral content of water on a land surface becomes more concentrated due to evaporation. Researchers have used methods like inverted microscopy, flow cytometry, pigment composition through HPLC, and a variety of molecular techniques like 16S rRNA gene sequencing, whole genome sequencing, MALDI-TOF, and FAME analysis to study microbial diversity and identify the members of a saltern community [33,34,35,36,37]. The polyphasic approach in culture-dependent identification of halophiles entails obtaining a pure culture, maintaining it on the desired medium, figuring out the best growth conditions, evaluating biochemical traits, figuring out the fatty acid composition of the cell membrane, describing genotypes, etc. [33, 38, 39, 40, 41, 42].

The group of microorganisms that live in a salty environment is known as the halophilic microbiome. This group includes bacteria, algae, fungi, viruses, and more. The protozoa are a group of eukaryotes that are frequently cast aside in studies of microbial diversity at high salt concentrations. However, microscopic examination methods make it simple to detect their presence [20, 33]. Some eukaryotes known as fungi can grow in environments with a lot of salt. Numerous fungi can survive in environments with little water, and they play a crucial role in saline and hypersaline ecosystems [33]. The green algae *Dunaliella* sp., a unicellular species, are found in hypersaline lakes all over the world [20, 34]. There have been reports of virus-like particles in the Dead Sea. The two most well-researched head-and-tail haloviruses are ΦH/ΦCh1 and HF1/HF2. It is still unknown how diverse haloviruses really are. At present, genome of very few haloviruses has been sequenced [33]. It's fascinating to study how haloviruses interact with their hosts.

On a variety of culture media, halophiles can grow. Essential nutrients are present in culture media, supporting the growth of microbial cells. Table 2 contains a list of crucial culture media needed for the isolation of halophiles.

Table 2 The culture media and their ingredients for isolating halophiles

Culture medium (with composition)	Reference
Starch Nitrate Medium (SNM) agar (g/L): NaCl 300.0, soluble starch 10.0, yeast extract 15.0, $MgSO_4 \bullet 7H_2O$ 20.0, KCl 2.0, $NaHCO_3$ 0.06, peptone 5.0, NaBr 0.23, KNO_3. H_2O 2.0, $FeCl_3$ $6H_2O$, $CaCl_2$ $2H_2O$ 0.36, $FeSO_4$. H_2O in traces and glycerol 5.0 (pH 7.2-7.4)	[9]
Abrram and Gibbons Medium (AGM) agar (g/L): NaCl 178.0, casamino acids 7.5, protease peptone 50.0, $MgSO_4$ $7H_2O$ 1.0, $CaCl_2$ $2H_2O$ 2.0, K_2HPO_4 7.5, $NaHCO_3$ 0.06, NaBr 0.23, peptone 5.0, yeast extract 10.0, Fe (NH_4) $(SO_4)_2$ 0.020 and $FeCl_3$ $6H_2O$ in traces (pH 7.2-7.4)	[9]
Halophilic Medium (HM) agar (g/L): Beef extract 1.0, Yeast extract 2.0, peptone 5.0, NaCl 300.0 (pH 7.2-7.4)	[9]
HM growth medium (g/L): NaCl (31, 81, or 181), $MgCl_2{\cdot}6H_2O$ (7), $MgSO_4{\cdot}7H_2O$ (9.6), $CaCl_2{\cdot}2H_2O$ (0.36), KCl (2), $NaHCO_3$ (0.06), NaBr (0.026), glucose (1), proteose-peptone (5), yeast extract (10) and agar (20)	[43]
JCM 168 growth medium (g/L): casamino acids (5), yeast extract (5), sodium glutamate (1), trisodium citrate (3), $MgSO_4{\cdot}7H_2O$ (20), KCl (2), NaCl (200), $FeCl_2{\cdot}4H_2O$ (0.036), $MnCl_2{\cdot}4H_2O$ (traces) and agar (20) (pH 7.2 before autoclaving)	[43]

Many halophiles have the capacity to produce hydrolytic enzymes that are crucial to industry. Producers of amylase, lipase, protease, DNase, and pullulanase include some species related to the *Salinivibrio*, *Halomonas*, *Chromohalobacter*, *Bacillus*, *Salibacillus*, *Salinicoccus*, and *Marinococcus* genera [6]. One of the sources of natural products is halophilic microorganisms. Microorganisms that produce carotenoids are primarily found in hypersaline lakes. Salinixanthine is a carotenoid that is produced by the halophilic bacterium *Salinibacter ruber* [6]. As food colouring agents, carotenoids are used in the food industry. The halophiles that were discovered in the marine environments of the Ratnagiri coast have antibacterial and antifungal properties [44]. Some halophiles can be used to make fermented foods like Thai fish sauce [6]. Biosurfactants and antibiotics are produced by some halophiles [45, 46]. The retinal protein bacteriorhodopsin is among the most well-known products derived from halophillic *Halobacterium* sp. [47]. From the respective strains of *Halobacterium cutirubrum*, *Halobacterium halobium*, and *Halobacterium salinarium*, the restriction enzymes HcuI, HhlI, and HsaI have been isolated. This suggests that halophiles may be used for genetic engineering. Halophiles are used in the degradation of hydrocarbons in the environment [47, 48]. Halophiles and their biomolecules have recently made advances and have potential uses in biomedicine, according to Corral et al. [49]. Maheshwari and Saraf have provided an illustration of halophile exploitation that is sustainable [50].

2. Conclusion

The ecosystems of saline and hypersaline lakes are completely reliant on halophiles, or salt-lovers. Many scientists have found halophiles in salt pans, salt marshes, salt lakes, hypersaline lakes, saline soils, and solar salterns. The compositions of some halophilic culture media, some halophilic microorganisms and their habitats, and a few halophile-related biotechnological applications have all been reviewed in this paper.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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Author’s short Biography

	**Dr. Mukundraj Govindrao Rathod (**Ph.D. & MH-SET): At Yeshwant College of Information Technology in Parbhani, Maharashtra, India, Dr. Mukundraj G. Rathod is the in-charge Principal. In addition, he heads this college's Biotechnology and Bioinformatics department. More than 77 research papers, including review articles in various peer-reviewed international and national journals and proceedings of conferences in various research fields, haveindeed been published by him. He has 300 citations in Google Scholar, an i10 index of 10, and a H index of 10, which is impressive. He has an amazing H index of 4, with 31 Scopus citations. He had given oral talks and posters at numerous conferences and seminars. He has also reviewed publications for a number of reputable scientific publishers. He had donated seven crucial industrial cultures to the National Center for Cell Science's Microbial Culture Collection in Pune, Maharashtra, India, for use by the general public. He is currently the lead researcher on a study supported by Swami Ramanand Teerth Marathwada University in Nanded through the Rajiv Gandhi Science and

	Technology Commission's application scheme for science and technology (Government of Maharashtra). According to the AD Scientific Index 2023, he recently held the 17th rank among the top 20 scientists at Swami Ramanand Teerth Marathwada University, Nanded, and its jurisdiction.
	**Mr. Jivan Munja Dhotare (**B.Sc. Agri., M.Sc.): Mr. J.M. Dhotare serves as an assistant professor at the College of Agricultural Biotechnology, Hatta. He has mastered the art of instructing students in microbial genetics, r-DNA technology, and related topics. He has taken part in numerous extracurricular and co-curricular activities and published three full-length research papers. He works as a programme officer for the National Service Scheme (NSS) in his college. Additionally, he attended the NSS State Adventure Camp in Chikhaldara, Amravati. He coordinates community service projects through NSS, such as blood donation, tree plantings, AIDS awareness campaigns, and awareness campaigns for intoxicants.
	**Prof. Dr. (Mrs.) Anupama Prabhakarrao Pathak (**Ph.D. & MH-SET): Dr. Anupama P. Pathak was previously serving as the School of Life Sciences' director and currently serving as the microbiology department's head at Swami Ramanand Teerth Marathwada University, Nanded. More than 200 research papers, including review articles, in various peer-reviewed international, national journals and conference proceedings were published by her. She has 774 citations in Google Scholar, an i10 index of 28, and a H index of 15, which is really impressive. She has an amazing H index of 8, with 239 Scopus citations. More than 110 16S rRNA gene sequences and numerous industrially significant bacterial cultures are among her contributions. She had finished two research projects in microbiology on extremophiles that were funded by the University Grants Commission of New Delhi. She is a member of the university's Microbiology Board of Studies. She is a renowned scientist who, according to the AD Scientific Index 2023, is ranking currently in ninth place of the top 20 scientists at Swami Ramanand Teerth Marathwada University, Nanded, and its jurisdiction.