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Research Article

HALOPHILIC PIGMENTS AS ACTIVE PHARMACEUTICAL COMPOUNDS; STRESS RESPONSE STRATEGIES, BIOTECHNOLOGICAL APPLICATIONS AND RECENT RESEARCH

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ABSTRACT

Halophiles are salt loving organisms thrive in hyper saline environments like salt pans, saltern pond brines, natural salt lakes and have a nearly neutral to slightly alkaline pH. These organisms have low nutritional requirements but shows resistance to high concentration of salt. Halophiles found in all three domains of life; Archaea, Bacteria, and Eukarya and categorized into three groups based on their NaCl requirement (slight (1-3%); moderate (3-15%); and extreme (15-30%)). Due to their existence in high salinity conditions, they provide number of novel elements. One among them is pigment. Halophiles producing pigment has various applications in field of biotechnology such as pharmaceuticals (antimicrobial, anticancer), neutraceuticals (antioxidant) and in food industries. This review provides an overview of habitat of halophiles, best sampling places to isolate halophiles to perform novel research and applications in various areas.

Keywords: Halophiles, Archaea, Salt Pans, Salinity gradient, Pigment, Applications.

INTRODUCTION

Halophiles are impressive group of extremophiles that persist in hyper-saline environments, often requiring these conditions for optimal growth. These microorganisms are found across all three domains of life: Archaea, Bacteria, and Eukarya. They manifest prominent metabolic diversity, being capable of processes such as phototrophy, fermentation, and methanogenesis, among others (Oren, 2002). Halophiles in their natural habitats i.e. hyper saline environment such as salt lakes, saline soils, and salted food products, regulates by maintaining low intracellular ionic concentrations while accumulating organic solutes to sustain osmotic balance (Ventosa *et al*., 1998). Notably, halophiles employ two main strategies for osmotic balance: the "high-salt-in" strategy, which involves importing potassium chloride and adapting intracellular proteins to function at high salt concentrations, and the "compatible solutes" strategy, which involves synthesizing or accumulating organic solutes that do not interfere with cellular functions (Oren, 2008).

Beyond their structural adaptations, halophiles possess distinctive metabolic pathways that enhance their biotechnological relevance. Notably, their capability to utilize hydrocarbons as both carbon and energy sources underscores their potential for bioremediation, especially in the remediation of saline environments polluted with resistant compounds (Margesin, R., Schinner 2001). Furthermore, halophiles are progressively recognized for industrial applications such as producing diverse metabolites and enzymes, including β-carotene and bacteriorhodopsin. These enzymes are widely used in food and cosmetics industries, along with the development of optoelectronic devices. In addition, halophilic enzymes have phenomenal stability under extreme conditions such as high temperatures. These organisms show activity in unfavourable environments due to the presence of compatible solutes which simulate them in industrial applications. (Kumar *et al*., 2016). Halophiles play a key role in keeping hypersaline habitats healthy with regulation of their nutrient cycle. Advanced methods like metagenomics aids in understanding the function of halophiles in harsh environments which leads to new discoveries (Oren, 2024).

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MATERIALS AND METHODS

Halophilic Environments

Halophilic environments include salt pans, saltern pond brines, and natural salt lakes which are salt rich habitats having unique ecosystem and functions as living lab for extreme microbial life.

Salt Pans

Salt pans are the lands covered by salt and other minerals. Diverse salinity range of halophiles finds favorable conditions in these high salinity environments. According to research halophiles form a bulk of bacteria communities in these high salinity environments which have above 10% salt concentration. (Rodriguez-Valera *et al*., 1981).

Saltern Pond Brines

Saltern pond brines are formed by salt water evaporation which are rich in halophilic population. Extreme halophiles dominated crystallizer and feeder ponds, especially Archaea like *Halorubrum* and *Haloquadratum*. Moderately halophilic species were demonstrated in cold ponds (Cusenza *et al*., 2025).

Natural Salt Lakes

Natural salt lakes consist of dissolved salts and minerals which provides optimal conditions for halophilic population. The dominant genera of salt lakes are *Halonotius*, *Halorubum*, and *Haloarcula*. Halophiles have varied metabolic pathways which sheds light on their roles in biogeochemical cycles and their role in environmental restoration. (Ding *et al*., 2025).

Characteristics of Halophiles

Salt Tolerance

Halophilies display varied modifications to survive in high salinity. To maintain integrity at salt concentrations, some halophiles such as those in the order *Halobacteriales*, use a "salt-in" strategy, my pumping potassium ions for balancing the osmotic pressure. This necessitates major adjustments to their intracellular enzyme machinery (Oren, 2008). As an alternative, many halophiles employ the "compatible solutes" technique, which involves the synthesis or ingestion of organic solutes such as ectoine, glycine betaine, and others in order to achieve osmotic equilibrium without interfering with enzyme activity. These organisms can adjust to a wider range of salt concentrations (Pflüger and Müller, 2004).

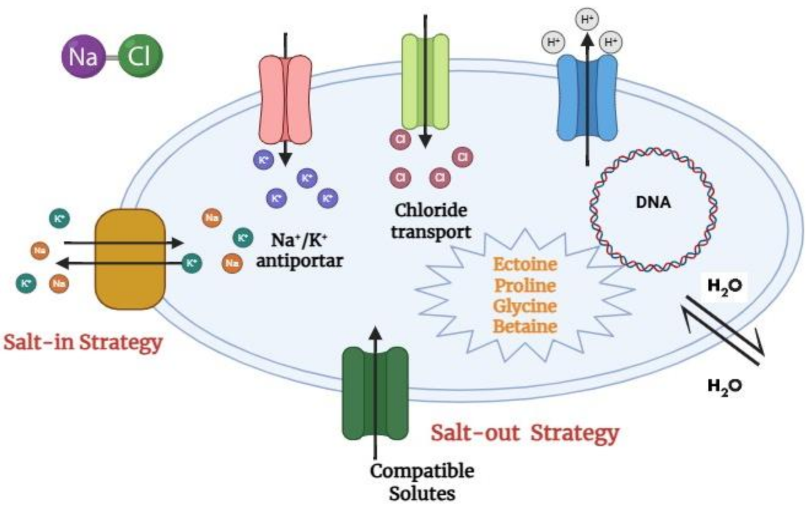

Figure 1. Diagrammatic view of Salt tolerance of halophiles. Reference is cited from Avigaël Ohayon *et al*.,2024 and Bhramar Dutta,2022. Diagram is developed using Biorendor.

Nutritional Requirements

Despite the metabolic diversity of halophiles, which include phototrophs, heterotrophs, and methanogens, even closely related species can differ greatly in how they use their substrates (Oren, 2002). Different carbon substrate usage profiles among halophiles isolated from various saltern environments show that the nutritional profiles of halophiles are frequently adapted to their particular environments, with microorganisms modifying their nutrient uptake mechanisms in accordance with the resources available (Rodriguez-Valera *et al*., 1981).

pH Preferences

Halophiles also exhibit varying pH tolerances, with some thriving in neutral pH while others prefer alkaline conditions. Many extremely halophilic archaea grow optimally at high salt concentrations and exhibit pH optima that are either neutral or strongly alkaline (pH 8.5 and above) (Bowers and Wiegel, 2011). This adaptability allows them to survive in varied saline habitats, further highlighting their evolutionary plasticity.

Classification of Halophiles

Taxonomic Distribution

Halophiles, organisms that thrive in high salt concentrations, exhibit considerable diversity across various taxonomic groups and adaptation strategies. Taxonomically, halophiles are found in all three domains of life: Archaea, Bacteria, and Eucarya. Within these domains, a variety of phylogenetic groups include halophiles. For instance, in the Bacteria, they are present in phyla such as *Cyanobacteria*, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Spirochaetes*, and *Bacteroidetes*. The Archaea, particularly the class *Halobacteria*, contain highly salt-requiring species (Oren, 2008; Oren, 1994).

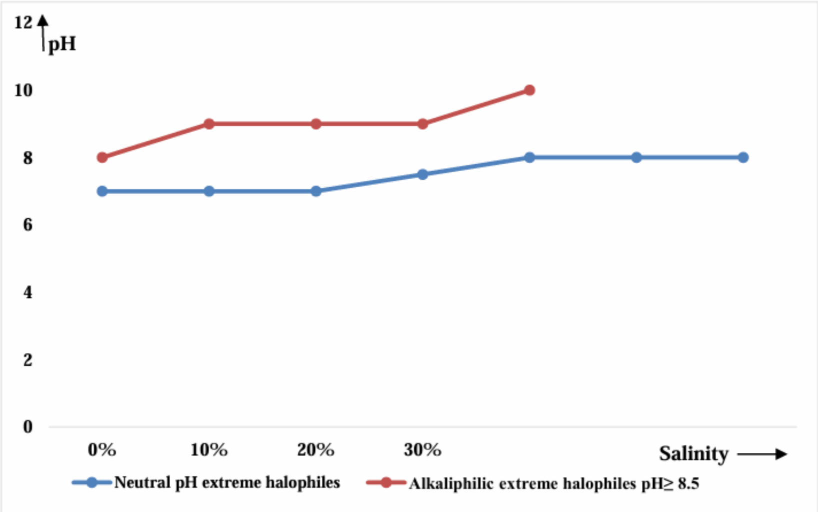

Figure 2. Graphical representation of pH and salinity preferences of Halophiles.

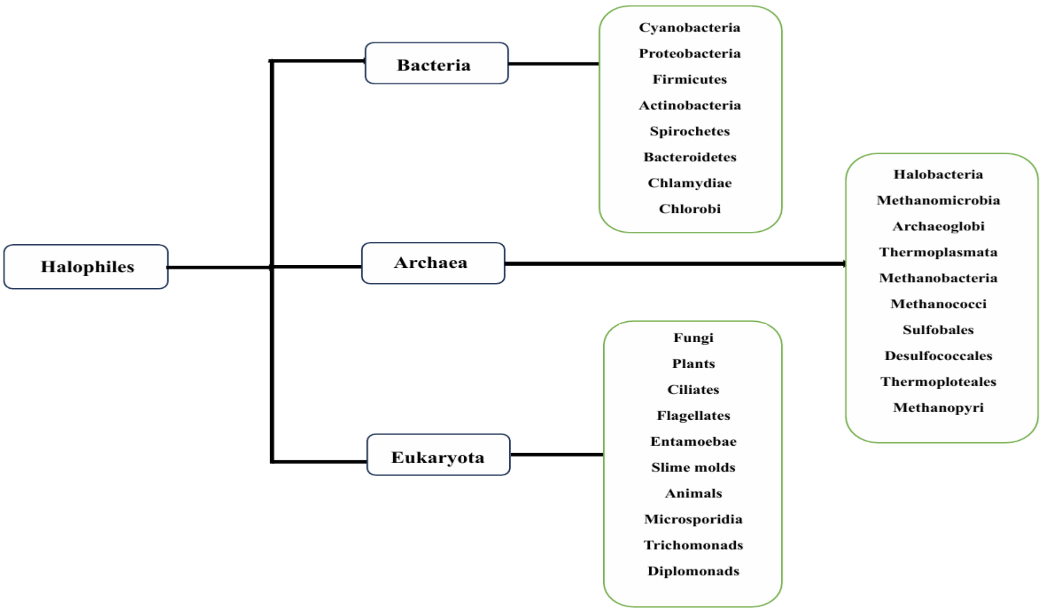

Figure 3. Schematic representation of taxonomic distribution of halophiles. Reference is cited from Mohamed Faraj Edbeib,2016.

Salt Requirement Categories

Halophiles are categorized based on their salt requirements into three groups: slight, moderate, and extreme halophiles.

Slight Halophiles

Slight halophiles thrive in environments with about 2-5% salt concentration. They show flexible physiological adaptations which activates them to survive at those concentrations. (Rodriguez-Valera, 1986).

Moderate Halophiles

Optimum salt concentration for moderate halophiles is 3%-15%. This category includes members from many microbial taxa and significantly multifarious. Moderate halophiles make the taxonomic diversity that include several taxa that are repeatedly adapted to changing salinity conditions. Salted food items or saline soils may provide suitable conditions for these microbes. (Ventosa *et al*., 1998; Rodriguez-Valera, 1986).

Extreme Halophiles

These types of halophiles survive in extreme salt conditions that is more than 20%. This category mostly found in Archaea especially the family of *Halobacteriaceae*. They thrive in halophilic environments like Dead Sea and salt ponds which provide extreme salinization. These extremophiles undergo adaptations to retain cellular function in these harsh environments. (Rodriguez-Valera *et al*., 1981; Oren, 1994).

Table 1. Classification based on salt requirement. Reference is cited from Bernard Ollivier *et al*.,1994.

Slight Halophiles (2-5%)	Moderate Halophiles (3-15%)	Extreme Halophiles (>20%)
Chromatium buderi	*Rhodospirillum mediosalinum*	*Ectothiorhodospira halophila*
Chloroherpeton thalassium	*Rhodospirillum salexigens*	*Ectothiorhodospira halochloris*
Ectothiorhodospira mobilis	*Ectothiorhodospira marismortui*	
Rhodobacter sulfidophilus	*Thiocapsa halophila*	
Pelodictyon phaeum	*Chromatium salexigens*	
Rhodopseudomonas marina	*Ectothiorhodospira abdelmalekii*	
Ectothiorhodospira vacuolate	*Rhodospirillum salinarum*	
Prostecochloris phaeoasteroidea		
Thiorhodovibrio winogradskyi		
Chlorobium chlorovibrioides		
Chromatium purpuratum		
Rhodobacter adriaticus		
Prosteocochloris aestuarii		
Chromatium vinosum HPC		
Lamprobacter modestohalophilus		

Adaptations to High Salinity

High salinity conditions provide harsh environment for cellular functioning. To survive and proliferate in those conditions halophiles adapted genotypic as well as phenotypic adaptations. These adaptations of both physiological and membrane are required for their survival and well-being (Dennis and Shimmin, 1997).

Membrane Adaptations

To survive under hyper saline conditions halophiles express genotypic and phenotypic modifications in their membrane composition. To reduce membrane pressure in saline conditions moderate halophiles and halotolerant increase anionic lipid content, significantly phosphatidylglycerol and glycolipids, that functions for osmoregulatory response. In addition with negatively charged groups such as sulfates, ether lipids present in extreme halophiles especially archaebacteria to amplify membrane stability under high saline environment. (Vreeland 1992).

Protein Adaptations

Halophilic proteins are enhanced with acidic amino acid residues, in high salt solutions resulting in an increased negative surface charge that involves in maintaining protein solubility and stability. This modification inhibits the accumulation due to salt-induced denaturation and enhances the hydration shell around protein. For example, the Cas9 protein in halophilic bacteria express numeric acidic residues and high intrinsic disorder, that enables function and stability in saline conditions by upholding a highly negative electrostatic surface potential (Debnath *et al*., 2025; Edbeib *et al*., 2016).

Osmotic Balance through Compatible Solutes

By synthesizing or aggregating organic compatible solutes like ectoine, proline, glycerol and trehalose osmotic balance of halophiles will be maintained. Cytoplasmic osmolarity will be maintained by these compounds with salinity resistance without interfering with cellular biochemical processes. In halophilic fungi like Aspergillus sydowii, glycerol aggregates predominantly under salt pressure, while trehalose is more abundant in salt-free environment. This selective association estimates the nuanced regulation of osmolytes based on salinity levels (Pérez-Llano *et al.*, 2020; Ventosa *et al.*, 1998).

Exopolysaccharide (EPS) Production

To maintain the integrity of cells from the detrimental effects of intense salinity many halophiles produce exopolysaccharides that develop extracellular polymeric substances. EPS undergoes many functions undergoing surface hydration maintenance, shield against desiccation and mechanical stabilization. Extremophiles producing EPS exhibit unique structural and functional properties beneficial for survival and have industrial applications as emulsifiers and stabilizers in unfavorable conditions. (Nicolaus *et al.*, 2010).

Cell Wall and Structural Modifications

In extreme saline environment specific halophilic fungi shows adaptations in cell wall composition. In addition induced cell wall thickness and lamellar architecture changes accumulated with reduced chitin content and augmented α- and β-glucan concentrations. Those structural changes stabilize cell integrity, helping tolerate osmotic pressure from hypersaline levels (Pérez-Llano *et al.*, 2020). Cumilatively, these membrane, protein, osmotic, extracellular, and structural adaptations facilitate halophilic microorganisms to sustain, function, and propagate in high saline conditions, underlying their ecological success and biotechnological relevance (Edbeib *et al.*, 2016; Nicolaus *et al.*, 2010; Pérez-Llano *et al.*, 2020; Russell, 1989).

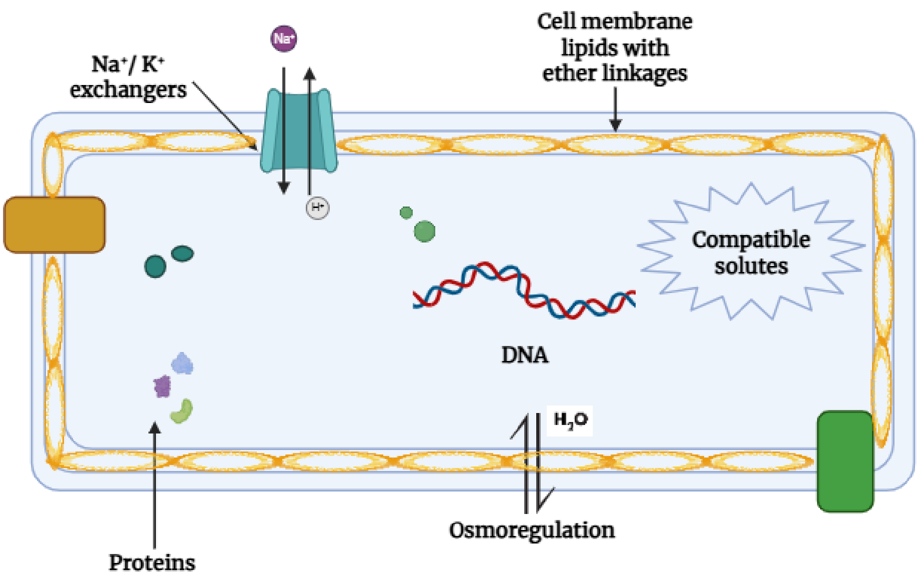

Figure 4. Diagrammatic view of adaptations of high salinity of halophiles. Reference cited from Ashok Bankar *et al.*,2022. Diagram is developed using Biorendor.

Halophilic Pigments

In addition to these adaptations halophilic species produce different pigments such as carotenoids and other chromoproteins that helps in ecological success. These pigments perform wide applications involving antioxidation, photoprotection and also osmoregulation. (Sabet *et al.*, 2009). Moreover, these pigments have vital role in biotechnological uses especially in industrial applications (Oren, 2002).

Carotenoid Pigments

Halophiles, especially extreme halophiles, produce a range of carotenoid pigments that facilitate to their characteristic reddish or orange coloration. Comparing to all these pigments bacterioruberins (C50 carotenoids) undergoes a vital role in protecting cells from oxidative damage and intense ultraviolet radiation in high saline conditions. In red halophiles like Halobacterium species both α- and β-bacterioruberins have been found, beside minor carotenoids alike to those identified in Rhodospirillum rubrum. Changes in growth conditions and culture age will not strongly affect the synthesis of these pigments. The production of these pigments often increases with culture age and is not strongly influenced by changes in growth conditions, suggesting their essential role in halophile physiological process (Baxter, 1960; Kushwaha *et al.*, 1974).

Isoprenoid and Other Pigments

Apart from carotenoids halophilic microorganisms also synthesize isoprenoid compounds such as lycopene, phytoene, squalene, and dihydrosqualene. In particular extreme halophilic microorganisms such as *Halobacterium* cutirubrum lycopene, a precursor to β-carotene in plants, accumulates in substantial amounts. These isoprenoid compounds involve in maintaining the membrane stability and functions as antioxidants, facilitating resistance for cells against hyper salinity and photodamage. Extreme halophiles produce retinal pigments whereas these retinal pigments are absent in moderate halophiles, reflecting differences in their ecological niches and modifications. (Kushwaha *et al*., 1974).

Biotechnological Applications of Halophilic Pigments

Halophilic pigments have a comprehensive biotechnological use in numerous industries due to their unique properties and beneficial impact. Following are the uses of halophilic pigments in the food, pharmaceutical, and nutraceutical industries:

Pharmaceutical Applications

Antimicrobial Properties

Halophilic microorganisms adapted to hyper saline conditions, expressing particular antimicrobial activity by synthesizing variety of bioactive compounds such as halocins, which are salt-dependent proteinaceous compounds predominantly synthesized by extremely halophilic archaea that stop closely related halobacteria leading to cell death and lysis through membrane disruption (Rodriguez-Valera *et al*., 1982). In addition, halophiles produce diverse secondary metabolites, enzymes, and lipases that exhibit antimicrobial activity effectively against a variety of microbial pathogens, including drug-resistant clinical strains (Santhaseelan *et al*., 2022; Thompson & Gilmore, 2023). Apart from antimicrobial properties halophilic compounds also undergo the alteration and disruption of microbial membrane permeability, inducing the lipid peroxidation causing cellular and DNA damage, suppressing of respiratory dehydrogenases resulting in microbial cell death. Antibiotic-resistant Haloarchaeal species will be inhibited by silver nanoparticles produced from halophilic microbial sources (Thombre *et al*., 2016).

Anticancer Properties

Halophiles synthesizing carotenoids associated with promising anticancer properties. Especially halophilic archaea such as *Haloarcula sp.* producing carotenoids exhibit highly active cytotoxic effects against breast cancer cell line (MCF-7). These halophilic pigments are morphologically equivalent to bacterioruberin, causes apoptosis by upregulating pro-apoptotic genes (CASP3, CASP8, BAX) and downregulating proliferation markers (MKI67, SOX2). This bidirectional regulation induces early and late apoptosis, emphasizing their viability as anticancer agents (Shahbazi *et al*., 2023). Halophilic microorganisms also synthesize variety of metabolites and enzymes that are capable for employing cancer therapeutics (Waditee-Sirisattha *et al*., 2016).

Nutraceutical Applications

Antioxidant Properties

Especially *haloarchaea* in halophilic microorganisms are well known for their capability for antioxidant activity, predominantly for their production of unique carotenoids like bacterioruberin and its derivatives. These C50 carotenoids act as a shield to the cells by facilitating resistance to oxidative stress induced by hyper salinity and vigorous UV radiation in high saline conditions (Giani *et al*., 2024). Bacterioruberin have potent radical scavenging activity and strong antihemolytic resistance to oxidative damage in erythrocytes that express exceptional antioxidant activity, inhibiting the widely accepted antioxidants such as β-carotene (Hou & Cui,2017). The adaptations undergone by the halophilic enzymes and biomolecules under hyper saline conditions leads their capability in biotechnological applications and as antioxidants suitable for industrial environments (Kumar *et al*., 2012).

Food Industry Applications

The unique enzymatic characteristics of halophiles leading their role in food industries. These organisms play a vital role in standard procedures like food fermentation and solar salt generation (Oren, 2010). Halophilic enzymes like lipases and proteases provide various food processing applications along with flavor modification and nutrient enhancement (Qiu *et al*., 2021). These enzymes exhibit vital role in generating omega-3 fatty acids and the enrichment of polyunsaturated fatty acids (PUFAs)and also these enzymes optimize cardiovascular health (Pérez *et al*., 2011). Moreover, these halophiles produce fermented foods, human supplements and leads to initiating new food items (Margesin and Schinner, 2001).

Optimal Sampling Locations

Natural saline habitats like salt lakes, salt mines, and salterns are perfect for sampling because halophiles flourish in situations with high salt concentrations. The bottoms of the Red Sea, Mediterranean Sea, and Gulf of Mexico contain deep-sea brines, which make them intriguing places to look for new microorganisms. Apart from their increased high salinity, they are anaerobic and form characteristically sharp brine-seawater interfaces, with some of the brines displaying significant increases in temperature and metal concentration. The ionic composition of the brines generally differs from that of seawater; they are anaerobic, and in some cases, the temperature can be elevated as well. The microbiology of Shaban Deep and other deep-sea brines in the Red Sea was discussed by Andre´ Antunes (Thuwal, Saudi Arabia). These sites, considered sterile in the past, have yielded several interesting microorganisms, including *Salinisphaera shabanensis* (a facultative anaerobe growing in a very large range of salt concentrations, from 1 to 28%), *Halorhabdus tiamatea* (a nonpigmented representative of the *Halobacteriales* that prefers an anaerobic life style, Flexi stipes *sinusarabici* (an anaerobe tolerating between 3 to 18% NaCl), and *Haloplasma* contractile (a contractile bacterium, phylogenetically equidistant to the Firmicutes and the Mollicutes) (Ma, Y., Galinski 2010). The Great Salt Lake in Utah is another prominent site, known for its exceptionally hypersaline conditions and unique microbial diversity (Tazi *et al*., 2014).

Future Prospects and Research Directions

The field of halophilic research presents numerous future prospects and research directions, particularly as our understanding of these organisms deepens and technology advances enable novel applications.

Genomic and Metagenomic Advancements

The application of next-generation sequencing technologies, including RNA sequencing and long-read technologies, is expected to enhance our understanding of halophilic communities. These technologies collab with genom-resolves metagenomics, yield complete penetration into halophilic microbiomes by facing hurdles like G + C content and intraspecific diversity (Uritskiy & Diruggiero, 2019).

Biotechnological Applications

Halophiles offer various biotechnological applications because of their sustainability in harsh environments. Halophiles like haloarchaeon Haloferax volcanii are applicable in biocatalysis, bioremediation and the biofuel sector. Biofilm development and bioreactor corrosion have to be resolved for the proper use of these microbes in industrial environments. (Haque *et al*., 2019).

Bioremediation and Environmental Applications

Halophiles are identified for having potentiality for bioremediation of heavy metal contamination in high saline habitats. Because of their sustainability in harsh environments, they are capable to decontaminate heavy metals and act as an ecofriendly agent. (Varshney *et al*., 2023).

Novel Compound Discovery

Halophilic environments are well known for novel compound discovery due to their environmental adaptations. Current research studies are saying that in halophilic environment novel beneficially relevant chemicals may be identified (Thompson and Gilmore, 2023).

Database and Bioinformatics Tools

Databases like HaloDom provides genetic and phenotypic data of halophilic species that list over 1000, helps research in biotechnology and comparative analysis of genomes (MB Ogaki, 2018).

CONCLUSION

Summary of this review is microbial taxa of these genera are interesting topic for current scientific investigation and industrial applications. Advanced techniques like genomics and metagenomics provided potentiality for researchers to study the genomic diversity of halophiles in deep. This leads to the discovery of biotechnological applications like discovery of novel compounds and production of enzymes and aiding to understand the deep biology. Beside these improvements there are obstacles in these studies such as the complications of halophilic adaptations and their disproportionate representation in genomic databases. These hurdles will be solved by sophisticated methods of bioinformatics and growing public datasets. Moreover, halophiles are spotlighted by their ecological importance such as potential applications in supportable environmental practices incorporating bioremediation and heavy metal decontamination.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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