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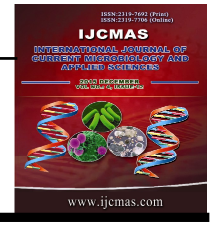

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Review Article

Halophiles-A Review

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A B S T R A C T

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Extremophiles, able to live in unusual habitats can serve as a potential source of novel microorganisms and their metabolites gain diverse applications and thus these microorganisms are extensively studied. Halophiles are a group of microorganisms that live in saline environments and in many cases require salinity to survive. Hypersaline habitats are tremendous sources of halophilic microorganisms and studies of these microorganisms are of special importance as they are capable of producing compounds of diverse industrial, pharmaceutical and biomedical potentials.

Introduction

Extremophiles are those microorganisms which require extreme environment for growth. The term extremophile was first used by MacElroy in 1974. Extreme environment is a relative term, since environments that are extreme for one organism may be essential for the survival of another organism. Extremolphiles are those microorganism which live in extreme environment like high or low temperature, salt concentration, pH etc. Extremophiles live under conditions that would kill most other creatures and many cannot survive in the normal global conditions. On the bases of their habitation these extremophiles can be categorized as: acidophiles, alkalophiles, halophiles, thermophiles and psychrophiles. Among extremophiles halophiles are those which are able to survive in high salt concentrations. These microorganisms inhabit the world's most saline environments, like hypersaline lake, solar saltern, evaporation pond, salt flats and tidal marine environments. Halophilic bacteria have the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts. On the basis of their halotolerance or optimum salt concentration wherein these organisms grow they are categorized as slight (2-5% NaCl), moderate (5-15% NaCl) or extreme halophiles (15-30% NaCl).

To survive in higher saline environment halophilic microorganisms use different types of strategies, they synthesize compatible solutes in cells or possess the transporters that help them to survive in such type of harsh condition. Osmoregulatory solutes such as potassium ion, glutamate, proline, ectoine and betaine have been reported in these bacteria (Madigan, 1999 and Galinski, 1993). Some halophiles produce acidic proteins that have the capacity to function in high salt concentration.

Examples of well-adapted and widely distributed extremely halophilic microorganisms include archaea for example *Halobacterium* sp. NRC-1, cyanobacteria such as *Aphanothece halophytica* and the green alga *Dunaliella salina*. Multicellular halophilic eukaryotic organisms include brine shrimp and the larvae of brine flies.

Occurence and Distribution

Halophiles can be found in areas where the concentration of salt is five times greater than that salt concentration of the ocean. Saline and hypersaline environments are broadly dispersed all through world either salt lakes (The Dead Sea, The Great Salt Lakes etc.) or salt mines [Salzbad-Salzetnan (Austria), Slanic, Turda, Praid (Romania), Wieliczka (Poland), Nakhlichevan (Azerbaidjan), Chon-Tous (Kirghizstan), Cave Berezniki in Perm(Russia), Solotvino (Ukraine) etc.]. These hypersaline environments are too harsh for normal life to exist, but a variety of microbessurvive.

These microorganisms produce different bioactive compounds like enzymes, pigments, solutes, metabolites and exopolysaccharides which have the ability to work in such type of saline conditions. These properties can be used for the development of techniques outlined essentially on the optimal condition of these biomolecules.

These properties could be exploited for the development of additional bio-industrial processes based on the optimal conditions of these biomolecules. To cope up with the high and often changing salinity of their environment, the aerobic halophilic bacteria, similar to all other microorganisms, need to balance their cytoplasm with the osmotic pressure exerted by the external medium (Madigan, 1999;Oren, 2008; Oren, 2010).

Important Characteristics Of Halophiles

Enzymes

Enzymes are catalysts which have potential applications in food, detergent formulations, metal recovery, leather processing and in several industries. Extremophile enzymes, especially extracellular, have been found to have potential industrial applications as they can survive and catalyze reactions in unusual environment.

Table 1 has listed different isolation sites from where halophilic microorganisms have been isolated and different enzymes which are assayed.

Nucleases

A nuclease (nuclease H) is reported from "*M. varians* subsp. *halophilus*" having both DNase and RNase activities. Another halophilic nuclease (an exonuclease, releasing 5′-mononucleotides from both DNA and RNA) was produced by *Bacillus halophilus* (Onishi*et al.*,1983).

Amylases

A few α-amylases were purified and characterized from halophilic microorganisms, *Acinetobacter* (Onishi*et al.*, 1980), *Halobacterium salinarum* (Goodand Hartman1970, Patel *et al.*, 1993), *M. varians* subsp. *halophilus*" (Kobayashi*et al.*, 1986), *N. halobia* (Onishi*et al.*, 1991), *Natronococcus amylolyticus* (Kobayashi *et al.*, 1992), *Halomonas meridiana* (Coronado *et al.*, 2000), *Haloferax mediterranei* (Perez-Pomares *et al.*, 2003)etc.

The enzyme from *Haloarcula* sp. S-1 showed high tolerance to various organic solvents (Fukushima *et al.*, 2005). The effect of ionic strength on the amylase activity, has also been reported at various ratios of Na^+ and Mg^{2+} concentrations (Enache *et al.*, 2009).

Proteases

Halophilic microorganisms produce proteases which can have novel applications (Margesin *et al.*, 2001) because of its high stability at saturated salt concentrations or organic solvent tolerance.

Halobacterium salinarum (Ryu *et al.*, 1994) produce an extra cellular serine protease that has potential to be used for peptide synthesis. The other serine proteases were isolated from *Natronococcu soccultus* (Studdert *et al.*,1997), *Natronomonas pharaonis* (Stan-Lotter *et al.*, 1999), *Natrial bamagadii* (Gimenez *et al.*,2000). Some other proteases were purified and characterized from *Natrial baasiatica* (Kamekura *et al.*, 1992) and *Haloferax mediterranei* (Kamekura *et al*,1992 and Kamekura *et al.*,1996) and other halophilic isolates (Norberg and Hofsten, 1969; Kamekura and Onishi, 1974; Kamekura and Seno, 1990).

In recent years, a number of studies have been conducted to characterize extracellular proteases from *Salinivibrio* genus (Lama *et al.*,2005 and Amoozegar *et al.*, 2007), *Pseudoalteromonas* sp. strain CP76 (Sa´nchez-Porro *et al.*, 2003), *Natrialba magadii* (Gime´nez *et al.*, 2000), *Halobacterium mediterranei* (Stepanov *et al.* 1992), *Bacillus clausii* (Kumar *et al.* 2004). There is also a report that a chymotrypsinogen B-like protease was isolated from the haloalkaliphilic archaeon *Natronomonas pharaonis* (Stan-Lotter*et al.*, 1999).

Lipases

Lipase is one of the most important hydrolytic enzymes with potential in various fields of pharmaceutical industry and agriculture. Various moderately or extremely halophilic microorganisms have been reported for the production of lipase which are also stable at high temperature - *Salinivibrio* sp. (Amoozegar *et al.*, 2008), *Natronococcus* sp. (Boutaiba *et al.*, 2006), haloarchaeal strains (Ozcan *et al.*, 2009) etc.

Cellulose-Degrading Enzymes

Bolobova *et al.*, (1992) first reported a cellulose-utilizing, extremely halophilic bacterium. The obligate anaerobic organism named *Halocella cellulolytica* is able to utilize cellulose as a sole carbon source. Another work has shown that many cellulose-utilizing extremely halophilic Archaea are present in subsurface salt formation (Vreeland *et al.*,1998). A preliminary work done by Cojoc *et al.*, (2009) on extracellular hydrolytic enzymes of halophilic microorganisms from subterranean rock salt revealed the presence of cellulose.

Pigments

Halophilic microorganisms are a great source of diverse natural products. Carotenoid pigments are one of these natural products responsible for the yellow, orange, red, and purple colors in a wide variety of plants, animals, and microorganisms (Li Z *et al.*,2012 and Cabral *et al.*,2011).Carotenoid pigments are particularly prominent in hypersaline environment. Red and orangish color of hypersaline habitats is due to the presence of pigmented microorganisms, including *Dunaliella*, rich in β-carotene, Haloarchaea whose main production is bacterioruberin, and halophilic bacteria, such as *Salinibacter ruber* producing a carotenoid called salinixanthin (El-Banna Aa*et al.*,2012 and Jehlicka *et al.*, 2013). An extremely halophilic archaeon isolated from Urmia Lake, *Halorubrum* sp. TBZ126 is reported as potential producer of carotenoids (Naziri *et al.*,2014).

Melanin is nearly a ubiquitous pigment having immense application potentials in the field of agriculture, cosmetics and pharmaceutical industries (photoprotection and mosquitocidal activity isolated from *Streptomycete*). Rani *et al.*,(2013) reported a halophilic black yeast, *Hortaea werneckii* which produced a diffusible dark pigment on potato dextrose agar. It also showed inhibitory activity against potential pathogens and activity was observed in *Salmonella typhi* and *Vibrio parahaemolyticus*.

Resistance to Antibiotics

The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal (Cowen, 2008). Pearson and Carol (2008) reported different antibiotics likes penicillin, erythromycin and gentamycin which used to be one of the important cures are now less effective because bacteria have become more resistant.

Halophilic bacteria isolated from different region were found resistant against different antibiotics :Halobactericeae (Hilpert, 1981), *Halomonas elongate* (Bonelo*et al*., 1984), *Bacillus cereus* SIU1 (Singh*et al.*, 2010).

Antimicrobial Activity

Foreshore soil of Daecheon Beach and Saemangeum Sea of Korea represents an untapped source of bacterial biodiversity and also that most actinobacterial isolates are capable of antibacterial and antifungal metabolite production. The halophiles isolated from Ratnagiri coastal area (marine environments) having antibacterial and antifungal activity. The antibacterial and antifungal assays of halophiles (protein crude extract) have shown that, the marine environments represent a potential source of new antimicrobial and antifungal agents (Todkar *et al.*, 2012).

Microbacterium oxydans and *Streptomyces fradiae* showed anibactrialactivity against all tested pathogenic bacteria and yeasts(Irshad *et al.*, 2013).Sawale *et al.*, (2014) collected **s**oil samples from coastal area of Arabic ocean (Mumbai) and reported antibiotics production from two bacterial strains *Bacillus pumilus* (NKCM 8905) and *Bacillus pumilus (*AB211228).

Applications

Biotechnological Application

Moderately halophilic bacteria have the potential for exciting and promising applications. Not only do many of them produce compounds of industrial interest (enzymes, polymers and osmoprotectants), but also they possess useful physiological properties which can facilitate their exploitation for commercial purposes. Here, we discuss some of the current industrial applications of these halophiles and emphasize some expected future developments.

Fermented Foods

Different fermented food products are prepared at a high salt concentration. For the preparation of such type of food products different microorganism are useful which can survive in such type of condition. For examplein preparation of Thai fish sauce (nam pla) the first halophilic archaeon isolated resembles *Halobacterium salinarum* (Thongthai and Suntinanalert, 1991 and Thongthai *et al*., 1992) and *Halococcus thailandensis* and *Natrinema gari* , were two new species recently isolated (Namwong *et al*.,2007 and Tapingkae *et al*., 2008). In fermentation of soy sauce halophilic lactococci (*Tetragenococcus halophilus*) are used as starters (Kushner*et al.,* 1998) .In the preparation of fermented liver sauce *Tetragenococcus muriatianus* is involved. *Halalkalicoccus jeotgali* is a novel isolate obtained from shrimp jeotgal (Roh *et al.*, 2007).

Halophilic Enzymes

Some of the halophilic bacteria produce hydrolytic enzymes which are stable at high salt concentration. These enzymes have diverse application in different fields. Some of these enzymes are proteases which have broad application in detergents, leather industry, food industry, pharmaceutical industry and bioremediation processes (Anwar and Saleemuddin, 1998 and Gupta *et al*., 2002). Bacteria of *Halobacterium* species like *Haloferax mediteranei*, *Natrial baasiatica*, *Natrial bamagadii*, *Natrono coccusoccultus* and *Natrono monaspharaonis* show protiolytic activity with potential industrial applications.

Amylases are used industrially in the production of high fructose corn syrup (hydrolysis of corn starch), the textile industry and are also used in laundry detergents. *Halomonas meridian*, *Haloarcula hispanica* and *Natronococcus amylolyticu* are some of halophilic bacteria from which amylases have been characterized.

Halophilc restriction endonuclease have diverse application. For the industrial production of the flavoring agent 5′-inosinic acid and 5′-guanylic acid Nuclease H produced by "*M. varians* subsp. *halophilus*" is useful (Kamekura M *etal.,* 1982). Some of endonucleases *HacI*, *HcuI*, *HhlI* and *HsaI* have been isolated and produced on commercial scale from *Halococcus acetoinfaciens*, *Halobacterium cutirubrum*, *H. halobium* and *Halobacterium salinarium* respectively.

Esterases and lipases are widely used as biocatalysts and are also useful ingredients in laundry detergents. The lipase from *Thermomyces lanuginosus* is commercially produced and sold as Lipolase by Novozymes, Denmark since 1989. An esterase from *Haloarculam arismortui* has also been purified and characterized.

Cellulase is used for commercial food processing in coffee and in textile industry, laundry detergents, in pulp and paper industry and they are even used for pharmaceutical applications (Cavaco- Paulo, 1998; Tolan and Foody, 1999 and Ikeda *et al*., 2006).

Compatible Solutes

To adapt to the osmotic stress and to keep up positive turgor pressure halophiles low molecular weight organic compounds. Some compatible solutes like glycine, betaine and ectoines, may be used as stress protectants and stabilizers of enzymes, nucleic acids, membranes and whole cells. The modern utilization of these compounds in enzyme technology (biosensor technology, PCR, and so forth) and for pharmaceuticals and cosmetics is a most encouraging field (Galinski and Tindall, 1992 and Ventosa*et al*., 1995).It may also enable the transfer of genes for salt and drought tolerance from moderate halophiles to agricultural crops, such as wheat, rice and barley, enabling them to grow in more saline soils.

Table.1 Microorganisms able to Produce Hydrolytic Enzymes Isolated from Different Hypersaline Environments

Isolation Site	Hydrolytic Activity Assayed	Most Abundant Hydrolytic Activity	Isolate Affiliation	References
Salterns in Almeria, Cadiz and Huelva (Spain)	Amylase protease lipase DNase Pullulanase	Amylase	*Salinivibrio* *Halomonas* *Chromohalobacter* *Bacillus-Salibacillus* *Salinicoccus* *Marinococcus*	Sánchez-Porro*et al.*,(2003)
Saltern in Huelva (Spain)	Lipase protease amylase nuclease	Amylase	*Halorubrum* *Haloarcula* *Halobacterium* *Salicola* *Salinibacter* *Pseudomonas*	Moreno*et al.*,(2009)
HowzSoltan Lake (Iran)	Lipase amylase protease xylanase DNase inulinase pectinase cellulase pulullanase	Lipase	*Salicola* *Halovibrio* *Halomonas* *Oceanobacillus* *Thalassobacillus* *Halobacillus* *Virgibacillus* *Gracilibacillus* *Salinicoccus* *Piscibacillus*	Rohban *et al.*,(2009)
Deep-sea sediments of the Southern Okinawa Trough (China)	amylase protease lipase DNase	Amylase	*Alcanivorax* *Bacillus* *Cobetia* *Halomonas* *Methylarcula* *Micrococcus* *Myroides* *Paracoccus* *Planococcus* *Pseudomonas*	Dang*et al.*,(2009)

			Psychrobacter *Sporosarcina* *Sufflavibacter* *Wangia*	
SlanicPrahova salt mine (Romania)	amylase gelatinase lipase protease cellulase xylanase	Lipase Protease	ND	Cojoc *et al.*,(2009)
Maharlu Salt Lake (Iran)	protease lipase	Not defined	*Bacillus* *Paenibacillus* *Halobacterium* *Aeromonas* *Staphylococcus*	Ghasemi *et al.*, (2011 a,b)
Saline desert "Indian Wild Ass Sanctuary" (India)	Amylase	Not defined	*Bacillus*	Khunt *et al.*, (2011)
Atacama Desert (Chile)	amylase protease lipase DNase xylanase pullulanase	DNase	DNase *Bacillus* *Halobacillus* *Pseudomonas* *Halomonas* *Staphylococcus*	Moreno *et al.*, (2012)
Arabal soil of west coast of Karnataka	Protease Amylase Lipase gelatinase inulinase	ND	*Virgibacillus* *Salinicoccus*	Jayachandra *et al.*,(2012a, b)
Balta Albă salt lake from Romania	Protease Amylase Cellulase Esterase Gelatinase	ND	ND	Neagu *et al.*, (2013)
Solar salterns of Orissa and West Bengal, India	amylase glutaminase asparaginase xylanase cellulose gelatinase inulinase caseinase pectinase urease lipase	glutaminase asparaginase lipiase caseinase	*Halomonas* *Cobetia* *Halococcus*	Biswas and Paul (2013)
Salt lakes of Romania	alanine-aminopeptidase α-glucosidase β –glucosidase	α –glucosidase β-glucosidase	ND	Păceşilă1 *et al.*, (2014)

Degradation of Toxic Compounds

Hypersaline waters and soils are often contaminated with heavy metals or other

toxic compounds from different sources. Hypersaline wastewaters are generated during the manufacture of chemicals such as pesticides, pharmaceuticals and herbicides and during oil and gas recovery processes. Conventional microbiological treatment processes do not function at high salt concentrations and therefore the use of moderately halophilic bacteria should be considered (Oren*et al.* 1992,1993).

Halophiles which are able to degrade hydrocarbon have been isolated from different sites like Great Salt Lake (Wardand Brock, 1978) and Antarctic saline lakes (McMeekin*et al.*, 1993). Treatment of hypersaline wastewaters containing phenol was reported by Woolard and Irvine (1992) using a biofilm of a moderately halophilic bacterium. *H. halodurans* reported to cleave benzoate and other aromatic compounds.DeFrank*et al,* (1991,1993) isolated halophilic bacteria, which were showing hydrolytic activity against several organophosphorus compounds. Halophiles belonging to the family *Halomonadaceae* have been recently isolated from highly saline sites contaminated with the herbicide 2, 4-dichlorophenoxyacetic acid, they were able to utilize chloroaromatic compounds as sources of carbon and energy.

Other Applications of Halophiles

Moderately halophilic bacteria may have various other uses in biotechnology, yet to be exploited. (i) Moderate halophiles could be used in the recovery of hypersaline waste brines derived from the olive oil industry and leather- or fur-curing processes. (iii) They may be screened for the production of bioactive compounds such as antibiotics. (iv) Biological surfactants derived from moderate halophiles may have a variety of applications in industry. Thus, Yakimov *et al.*, (1996) recently isolated a moderate halophile which synthesizes a novel glycolipid belonging to a powerful novel class of biosurfactants.(vi) Many moderate halophiles produce orange or pink colonies, probably due to the production of carotenoids as a protective mechanism against photooxidation processes. Carotenoids have major applications in the food industry as food-coloring agents and as additives in health food products. Therefore, investigations of the utilization of moderate halophiles as producers of carotenoids could be of great interest.

In conclusion, comparative studies of micro flora in the extreme environments results in better understanding of the ecosystem and can benefit in designing the applications. Hypersaline environments represent a valuable source of different bioactive compounds with great economical potential in industrial, agricultural, chemical, pharmaceuticals and biotechnological applications The microbial diversity can prove to be a valuable future resource in various industrial and biotechnological processes. Such microbes can also be used as a source of gene(s) that can increase salt tolerance in different crop species through genetic transformation.

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