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Introduction to Halophiles

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ABSTRACT

Halophiles are organisms which multiply in salty, marshy, hypersaline environments, such as salt lakes, solar salterns and salt mines. Halophiles are classified in different categories like halophilic bacteria, halophilic fungi, halophilic algae, halophilic archaea, halophilic protozoa.

Such salt tolerant organisms are widely used for the production of valuable enzymes, pigments, retinal proteins, antibiotics and bioremediation of toxic waste, preservation of perishable foods and other materials. The aim of this review is to provide an overview of halophiles, their types, their current and future biotechnological and environmental uses.

Keywords: *Hypersaline environments; Halophilic bacteria; Environmental uses.*

INTRODUCTION

Extremophiles are highly diverse in nature. They are found in extreme environments like high or low pH, high or low temperature, high salinity, high pressures. They are able to live in unusual habitats, can potentially serve in a variety of industrial applications. Extremophiles have evolved unique properties, which can provide significant commercial opportunities. All 3 domains of life are included in extremophiles - the Archaea , Bacteria and Eukarya. They are divided in 5 categories

- Thermophiles ,
- Acidophiles,
- Alkalophiles,
- Psychrophiles ,
- Halophiles.

This clearly indicates the nature of habitats used by these microorganisms (Austain, 1988).

HALOPHILES

Halophilic microorganisms thrive in salty environment. The ecological characteristics of halophiles have been extensively investigated (Jiang *et al*., 2007) in saline and hypersaline habitat. Halophiles are found in arid, coastal and even in deep sea location, as well as in artificial slatterns. Halophiles are salt loving, they are adapted to high osmolarity and can grow in high saline environments. They include both prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturating effects of salts. Osmoregulatory solutes such as potassium ion, proline, ectoine, betaine have been reported (Galinski., 1993) in halophiles.

Examples of well adapted and widely distributed extremely halophilic microorganisms include archaea for example *Halobacterium* sp. NRC – 1, cyanobacteria such as *Aphanotheca haophytica* and the green alga *Dunaliella salina*., multicellular halophilic eukaryotic organisms include brine shrimps and larva of brine flies.

Halophilic archaeal cell membranes contain phospholipids composed of branched isoprene units linked to glycerol by an ether group, where as bacterial and eukaryal membranes have fatty acids linked to glycerol by an ester bond. These archaeal membranes are less permeable to ions and more resistant to high salts.

To avoid excessive water loss, halophiles have developed two distinct strategies to increase the osmotic activity of their cytoplasm.

(i) First strategy is cytoplasmic accumulation of molar concentrations of KCl which is called as “high salt-in” strategy.

(ii) Second strategy is the cytoplasmic accumulation of organic compounds, called as compatible solutes. These compatible solutes can be taken up from the environment by specific transport systems or biosynthesized by the halophilic organism. This strategy is called “low-salt, organic-solute-in”.

Presence of high intracellular salt in halophiles, requires adaptations of the whole enzymatic machinery of the cell. The cytoplasmic accumulation of molar concentrations of KCl results in a sharp decrease in the distribution of protein isoelectric points. They have also developed resistance to various toxic substances including heavy metals.

Adaptations and the unique physiologies of these organisms are explored for various biotechnological applications.

The halophiles have some advantages as compared to other microorganisms

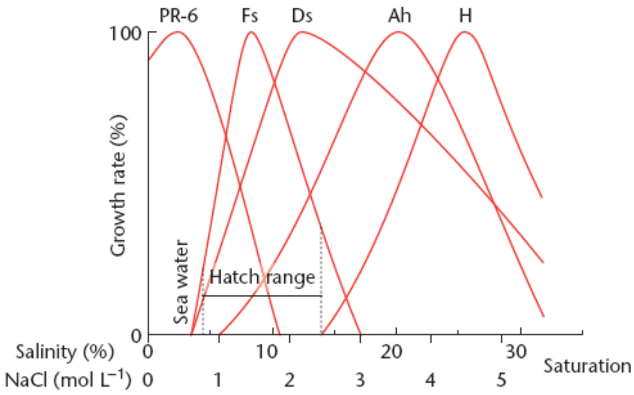
1) High potential for genetic manipulation ,
2) They are easy to grow ,
3) Easy to maintain in the laboratory,
4) The necessity for aseptic conditions is decreased to a minimum.

CLASSIFICATION OF HALOPHILES

Kushner and Kamekura (1988) defined several categories of halophilic microorganisms

1) Non halophiles - optimal growth is below 1% salt.
2) Halotolerant - optimal growth occur below 1 % but growth is also observed at higher salt concentrations.
3) Slight halophiles - grow best between 1% and 3% salt
4) Moderate halophiles - grow best between 3 % to 15 % salt
5) Extreme halophiles - grow optimally above 15% salt

Moderate halophiles and extreme halophiles generally predominates in hypersaline environments.



Salt-tolerance of halophilic organisms. Relative growth rate is plotted against both per cent salinity and NaCl concentration. The five microorganisms depicted are *Synechococcus* sp. PCC7002 (PR-6), a slightly halotolerant cyanobacterium, *Fabrea salina* (Fs), a moderately halophilic protozoan, *Dunaliella salina* (Ds), a halophilic green algae, *Aphanothece halophytica* (Ah), an extremely halophilic cyanobacterium and *Halobacterium* sp. (H), an extremely halophilic archaeon. The salinity of seawater and the hatch range for brine shrimp are noted.

TYPES OF HALOPHILES

1) Haloarchaea

They are members of the archaeal domain , they are also called halophilic archaea, and formerly halobacteria .They have recently been proposed to be composed of two families and includes about three dozen genera , metagenomic study of a solar saltern showed the occurrence of a major new phylotype, called nano haloarchaea, with small cells (50.8 um) . Other non culture based studies have suggested that novel species similar to haloarchaea may occur in the human gastrointestinal tract .

2) Halophilic bacteria

Halophilic bacterium are gram negative or gram positive, aerobic or facultative aerobic in nature. Halobacterium strains isolated in the mid- twentieth century from salted fish and meat from Europe and North America (Das Sarma *et al*., 2010). These halobacterium are amino acid-utilising facultative aerobes, they require a number of growth factors and slightly elevated temperatures (38– 45.8 C) for optimal growth. Some of them have distinctive features such as gas vesicles, purple membrane and red-orange carotenoids . Yancey, 2005 reported that many halophiles are facultative anaerobes and grow utilising respiration of dimethoxyl sulfoxide and trimethylamine –N -oxide a salt water fish osmolyte , fermentation of different sugars, breakdown of arginine and light energy, mediated by retinal pigments.

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TABLE 1

Main characteristics of moderately halophilic gram-negative members of the *Halomonadaceae*[a]

Feature	*H. elongata* (357)	*H. subglaciescola* (74)	*H. halodurans* (115)	*H. halmophila* (54)	*H. eurihalina* (200, 266)	*H. halophila* (269)
Morphology	Rods	Rods	Rods	Rods	Short rods	Rods
Size (μm)	ND	0.5–1.1 × 5–10	0.4–0.6 × 1.5–2.0	0.3–0.6 × 0.9–1.3	0.8–1.0 × 2.0–2.5	0.5–0.7 × 1.5–2.0
Pigmentation	None	Cream	None	Cream	Cream	Cream
Motility	+	+	+	+	−	+
Facultative anaerobe	+	−	−	−	−	−
Oxidase	+	+	+	+	−	+
NaCl range (%)	3.5–20	0.5–20	3.5–20	0.5–20	5–20	2–30
NaCl optimum (%)	3.5–8	ND	8	ND	7.5	7.5
Temp range (°C)	15–45	0–25	4–37	ND	15–45	15–45
pH range	5–9	5–9	5.5–8.5	ND	5–10	5–10

Feature	*H. salina* (53, 336)	*H. halodenitrificans* (53, 159)	*H. variablis* (53, 65)	*H. canadensis* (128)	*H. israelensis* (128)	*H. pantelleriense* (290)	*Chromohalobacter marismortui* (344)
Morphology	Short rods	Short rods	Curved rods	Rods	Rods	Rods	Rods
Size (μm)	0.7–0.8 × 2.0–2.5	0.5–0.9 × 0.9–1.2	0.5–0.8 × 1.0–3.0	ND	ND	0.4–0.7 × 1.4–2.6	0.6–1.0 × 1.5–4.0
Pigmentation	Yellowish or cream	Cream	Cream	White	Cream	Cream-pink	Brown-yellow
Motility	−	−	−	+	ND	+	+
Facultative anaerobe	−	−	−	−	−	−	−
Oxidase	+	+	+	−	−	−	−
NaCl range (%)	2.5–20	3–20	7–28	3–25	3.5–20	1.2–15	1–30
NaCl optimum (%)	5	5–9	10	8	8	10	10
Temp range (°C)	15–40	5–32	15–37	15–30	15–45	10–44	5–45
pH range	6–10	ND	6.5–8.4	5–9	5–9	7.5–11	5–10

[a]+, positive; −, negative; d, differs among strains; ND, not determined.

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TABLE 2

Main characteristics of other aerobic or facultatively anaerobic gram-negative moderately halophilic bacteria[a]

Feature	*Salinivibrio costicola* (91, 201)	*Pseudomonas halophila* (65)	*Flavobacterium gondwanense* (52)	*Flavobacterium salegens* (52)	*Arhodomonas aquaeolei* (4)	*Spirochaeta halophila* (103)	*Dichotomicrobium thermohalophilum* (124)
Morphology	Curved rods	Rods	Rods	Rods	Short rods	Helicoidal	Prosthecate
Size (µm)	0.5 × 1.5–3.2	0.8–1.0 × 1.5–5.0	0.4–0.8 × 1.7–11.7	0.5–0.8 × 1.2–11.5	0.8–1.0 × 2.0–2.5	0.4 × 15–30	0.8–1.8 × 0.8–2.0
Pigmentation	Cream	Reddish-brown	Orange	Yellow	None	Red	Reddish-brown
Motility	+	+	–	–	+	+	–
Facultative anaerobe	+	–	–	–	–	+	–
Oxidase	+	+	+	+	+	ND	+
NaCl range (%)	0.5–12	0.1–20	0–5	0–20	6–20	0.5–8	0.8–22
NaCl optimum (%)	10	5	5	5	15	5	8–14
Temp range (°C)	5–45	4–37	0–30	ND	20–45	25–40	20–60
pH range	5–10	4.5–9.6	5–9	5–9	6–8		5.8–9.5

[a]+, positive; −, negative; d, differs among strains; ND, not determined.

TABLE 3

Main characteristics of aerobic gram-positive moderately halophilic bacteria[a]

Feature	*Halobacillus*			*Bacillus*	
	H. halophilus (33, 315)	*H. litoralis* (315)	*H. trueperi* (315)	*B. halophilus* (342)	*B. salexigens* (89)
Morphology	Coccoid	Rods	Rods	Rods	Rods
Size (µm)	1.0–2.0 × 2.0–3.0	0.7–1.1 × 2.0–4.5	0.7–1.4 × 2.0–4.5	0.5–1.0 × 2.5–9.0	0.3–0.6 × 1.5–3.5
Pigmentation	Orange	Orange	Orange	None	None
Spore shape	S	E/S	E/S	E	E
Spore position	C/ST	C/ST	C/ST	C	ST/C
Sporangium swollen	–	ND	ND	–	+
Motility	+	+	+	+	
Facultative anaerobe	–	–	–	–	–
Oxidase	+	+	+	+	+
NaCl range (%)	2–20	0.5–25	0.5–30	3–30	7–20
NaCl optimum (%)	10	10	10	15	10
Temp range (°C)	15–40	10–43	10–44	15–50	15–45
pH range	7–9	6–9.5	6–9.5	6–8	6–11

M. halophilus (111, 189)	*M. albus* (111)	*S. roseus* (344)	*S. hispanicus* (188, 345)		
Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
1.0–1.2	1.0–1.2	1.0–2.5	1.0–2.0	ND	0.5–0.8
Yellow-orange	None	Pink red	Reddish-orange	None or yellow	White
–	–	–	–	–	–
–	–	–	–	–	–
–	–	–	–	–	–
+	+	–	–	–	–
–	–	–	–	–	+
–	+	+	+	+	–
2–25	2–25	0.9–25	0.5–25	3–25	1–25
10	10	10	10	10	7–10
15–37	15–37	15–40	15–37	20–40	15–40
ND	ND	6–9	5–9	5–10	5–9.6

[a]S, spherical; E, ellipsoidal; C, central; ST, subterminal. +, positive; −, negative; d, differs among strains; ND, not determined. MK-9, MK-8, MK-7, MK-6, menaquinone with nine, eight, seven, and six isoprene units, respectively. *m*-Dpm, *meso*-diaminopimelic acid.

TABLE 4

Main characteristics of moderately halophilic actinomycete species[a]

Feature	*Actinopolyspora*		*Nocardiopsis*	
	A. mortivallis (374)	*A. iraquiensis* (292)	*N. lucentensis* (371)	*N. halophila* (6)
Spore chain morphology	Short (no more than 10)	Short (no more than 15)	Short	Very long
Pigmentation	Yellowish white	Yellow-brownish	Yellowish brown	Yellow-coral red
Spore	Oval to cylindrical	Spheroidal	Cylindrical	Cylindrical
NaCl range (%)	5–30	5–20	ND	5–20
NaCl optium (%)	10–15	10–15	5–10	15
Temp range (°C)	10–50	16–40	ND	16–40
Acid production from:				
Glucose	+	ND	+	–
Mannitol	–	+	+	+
Xylose	+	–	–	+
Nitrate reduction	–	ND	+	ND

[a]+, positive; −, negative; ND, not determined. MK-10 (H_8, H_6, and H_4), octa-, hexa-, and tetrahydrogenated menaquinones with 10 isoprene units; MK-9 (H_4), tetrahydrogenated menaquinone with 9 isoprene units.

3) Halophilic fungi

Halophilic fungi are adapted to extreme conditions of temperature, pH and salinity. *Mangicolous* filamentous fungi have been isolated from different parts along the Indian west coast .

Halophilic fungi are ubiquitous and usually colonize on diverse range of substrates. Earlier studies reported fungi belongs to ascomycota generally found in extreme environments and mainly inhabit saline soils, sea water, mangroves and salty marshes.

A halotolerant yeast *Debaryomyces hansenii*, isolated from seawater can grow aerobically in salinities of upto 4.5M NaCl, and has been studied extensively by genome sequencing and transcriptomic analysis . It produces glycerol compatible solute during logarithmic phase and arabitolin during stationary phase of growth. *Hortaea werneckii*, a melanised fungus, was isolated from hypersaline waters of solar saltern sand its osmoresponsive genes have been identified by transcriptomic methods (Lenassi *et al*., 2007).

4) Halophilic protozoa

A large number of protozoa exist in hyper saline environments, but few of them have been extensively described. One moderately halophilic ciliate, *Fabrea salina*, has been isolated from several saline lakes from Africa to Australia. Although in freshwater, protozoa regulate osmotic pressure with contractile vacuoles that expel water, their mechanism of osmoregulation in hypersaline brine has not yet been investigated.

5) Halophilic algae

Dense populations of green algae are reported at moderately high salinities (1–3.5M NaCl) (Javor, 1989). Dunaliella species, for example *Dunaliella parva* and *Dunaliella viridis*, are ubiquitous and are the main source of food for brine shrimp and the larvae of brineflies. Most species are moderate halophiles, with only a few extremely halophilic species, for example *Dunaliella salina* and *Asteromonas gracilis*, they are capable of slow growth at upto saturated NaCl concentrations. The algae predominantly use polyolsas compatible solutes. *Dunaliella salina* synthesises glycerolin response to osmotic stress. (Jiang , 2007).

APPLICATION OF HALOPHILES

Halophiles produces novel biomolecules that are of commercial interest, some of these applications of halophiles isolates are

A) Retinal proteins

The ability to convert light to chemical energy in a nonchlorophyll system was first discovered in the haloarchaea .The apoprotein responsible for this, bacterioopsin, is combined with a retinal protein to make bacteriorhodopsin, which is then organized into a two-dimensional crystalline array in the purple membrane of haloarchaea. Bacteriorhodopsin from halobacterium species is marketed for light sensors, nonlinear optics. It has been suggested that bacteriorhodopsin could be use to give sight to industrial robots. (Das Sarma *et al* ., 2010)

B) Compatible solutes

Halophiles produces compatible solutes or osmolytes that maintain the stability of biomolecules. Osmolytes are usually-glycine-betaine, actoine or sugars and polyols-sucrose, trehalose or glycerol. Halotolerant yeast and green algae accumulate polyols, while halophilic bactiria accumumulates zwitterions. Green algae *Dunaliella salina* is good commercial source of glycerol.

C) Nutritional applications

Halobactirica, Halococci and *Natrococci* have been isolated from sources included fermented food. Green singal celled alga *D. salina* accumulates carotenoids at high salinity with an optimum yield of beta carotene around 24% NaCl. Beta carotene is lipid and oil soluble product with industrial applications like food coloring, baked food, emulsifiers. Beta carotene is pro vitamin A. *D. salina* produces carotene and xanthophylls (Lamers *et al* . , 2008)

D) Halophilic enzymes

Sum of the halophilic bacteria produces hydrolytic enzymes such as lipases (Ozcan *et al* .,2009) , proteases (Schinner *et al* . , 2001) and amylases (Onishi *et al* ., 1980) Protease represent one of the three largest group of industrial enzyme and find application in detergents, leather industry, food industry, pharmaceutical industry (Gupta *et al* .,2002). Halophiles may be useful for industrial production of flavouring agent 5'- inosinic acid and 5'- guanylic acid (Kamekura *et al* .,1982). Isolation of novel *Bacillus* species such as *Bacillus salexigens* which produces extra cellular nuclease in saline medium reported by (Garabito *et al*.,1997).

E) Degradation of toxic waste

Heavy metals, pesticides, pharmaceuticals, herbicides or other toxic compounds from anthropogenic sources generally contaminate salty environment. Some 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). Hydrocarbon-degrading moderate halophiles have been isolated from a variety of environments, including the Great Salt Lake (Ward *et al.*, 1978) and Antarctic saline lakes . Woolard and Irvine (1992) reported the utilization of a biofilm from a moderately halophilic bacterium isolated from a saltern at the Great Salt Lake, Utah, for the treatment of hyper saline waste waters containing phenol. Batch biofilm reactor, were used to remove phenol from a waste containing 15% salt. Some aromatic compounds , like benzoate can be degraded by *H. halodurans* by cleavage of aromatic rings. A moderately halophilic bacterium isolated from a hyper saline spring in Utah degrades highly toxic organo phosphorus compounds. *Alteromonas* strain was identified by (DeFrank *et al.*, 1991) which grew at 2 to 24 % salt.. The enzyme organophosphorus acid anhydrase, was purified and characterized. Five additional halophilic bacteria, showing hydrolytic activity against several organophosphorus compounds and related chemicals, were isolated (De Frank *et al.*, 1993). Such enzymes may have considerable potential for the decontamination and mineralization of chemical warfare agents.

Some moderate 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. One of the isolates, strain I-18, showed high activities of catechol 1, 2-dioxygenase, muconatecyclo isomerase, and dienelactone hydrolase at about 1.0 M NaCl and pH 8.4 to 9.4. This strain was also able to utilize other aromatic compounds including benzoic acid, 3-chlorobenzoic acid, and 4-chlorophenol.

F) Fermented food

Tetragenococcus strains are involved in the fermentation of soy sauce. In soy sauce manufacture, ground wheat and soy grains are suspended in water with about 19% NaCl and incubated for up to 9 months in the dark. Halophilic *lactococci* , *Tetragenococcus halophilu* are used as starters for the fermentation and typically develop densities of up to 10^8 CFU/ml in soy sauce mash with about 3 M NaCl. *Tetragenococcus muriatianus* is involved in the preparation of fermented liver sauce. In the preparation of Thai fish sauce (nampla) , a food condiment widely used in South East Asia , moderate halophiles and halotolerant bacteria are used (*Bacillus* sp., coryneform bacteria) most of these tolerating up to 20 to 30%. Extremely halophilic red archaea are also found during the process. (Thongthai *et al.*, 1991).

G) Other Applications

Some additional potential applications of moderately halophilic microorganisms has been found these are:-

(i) Moderate halophiles can be used to remove phosphate from saline environments, as a cheaper alternative to chemical approaches (Ramos-Cormenzana, 1991).
(ii) 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) Halophiles are screened for the production of bioactive compounds such as antibiotics.
(iv) Biological surfactants are also derived from moderate halophiles (Yakimov *et al.,* 1996) recently isolated a moderate halophile which synthesizes a novel glycolipid belonging to a powerful novel class of biosurfactants.
(v) New restriction endonucleases and other enzymes from hypersaline habitats and will be discovered and exploited.
(vi) Some 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.

PRODUCT ISOLATED FROM HALOPHILIC MICROOGANISMS

A) Enzymes

Enzymes are catalyst which have tremendous applications, in metal recovery, food , detergents . Halophilic enzymes especially extra cellular, have potential industrial applications. . Halophilic amylases ere characterized from a moderately halophilic *Actinobacter* (Onishi *et al* ., 1980), *N halobia* (Onishi *et al* ., 1991), *M.varians* subspc. Halophilus (Kobayashi *et al* ., 1986), and other micrococcus isolates (Onishi . ,1972).

- **Proteases**

Halophytic microorganisms produces proteases with high stability at saturated salt concentrations or organic solvent tolerance which can have novel applications (Schinner *et al.,* 2001). Protease isolated from halobacterium has been used in detergent and food industries.

An extra cellular serine protease has potential to be used for peptide synthesis, particularly those containing glycine, produced from *Halobacterium salinarum* (Ryu *et al.*, 1994).

Another protease produced by *Natrial baasiatica* and *Haloferax mediterranei* (Kamekura *et al* .,1992, Kamekura *et al.*, 1996) was purified and characterized.

Some other serine proteases were isolated from *Natronomonas pharaonis* (Lotter *et al.*, 1999), *Natrial bamagadii* (Gimenez *et al.*, 2000), *Natronococcu soccultus* (Studdert *et al.*, 1997).

The biochemical characterization and modification of these enzymes based on their cloned genes (Kamekura *et al* . , 1992, 1996; Shi *et al* . , 2006) will help to improve the understanding halophilic protease and contribute to elucidating mechanisms for their activation and extracellular secretion (De Castro *et al.,* 2006).

- **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, which are relatively stable at high temperature *Salinivibrio* sp. (Amoozegar *et al.,* 2008), *Natronococcus* sp. (Boutaiba *et al.*, 2006), haloarchaeal strains (Ozcan *et al.*, 2009) have been shown to produce lipases.

- **Cellulose-degrading enzymes**

Bolobova *et al.*, (1992) reported 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).

- **Nucleases**

“M. varians subsp. halophilus” produces a nuclease (nuclease H) when grown in 1 to 4 M NaCl or KCl. The purified enzyme has 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).

A preliminary work on extracellular hydrolytic enzymes of halophilic microorganisms from subterranean rock salt revealed the presence of cellulase (Cojoc *et al.*, 2009).

- **Amylases**

A few α-amylases having similar functional properties were purified and characterized from halophilic microorganisms, *Haloferax mediterranei* (Perez-Pomares *et al.*, 2003), *Halobacterium salinarum* (Good *et al., 1970) Natronococcus amylolyticus* (Kobayashi *et al.*,1992), *Halomonas meridiana* (Coronado *et al.*, 2000) etc., .

The enzyme isolated from *Haloarcula* sp. S-1 which differentiated it from other haloarchaeal α-amylases usually active under high salt concentrations and generally inactive in the absence of salt showed a relatively high tolerance to various organic solvents (Fukushima *et al.*, 2005).

Enache *et al.*, 2009 reported the effect of ionic strength on the amylase activity, at various ratios of Na^+ and Mg^{2+} concentrations.

B) Pigments

- **Prodigiosin**

Serratia lebsiella produces a red color pigment called as prodigiosin. The red pigment produced by the isolate *Serratia marcescens* IBRL USM 84 was extracted and analyzed and Isolates from marine bacteria have been proven to contain active compounds, prodigiosin (red pigment), violacein (violet pigment) and pyocianin (blue-green pigment) these pigments contain active compounds having anitimicrobial activity, antiviral, antitumor, antiprotozoa, antioxidant, anticancer and much more (Kim *et al*., 2010; Solieve *et al*., 2011).

Red pigment has higher antimicrobial especially antibacterial activity followed by orange, yellow and green (Solieve *et al*., 2011).

Marine bacterias that have been reported to produce red pigment with prodigiosin or antibacterial activity are *Serratia marcescens* (Solieve *et al*., 2011) *Streptomyces* sp. , *Pseudomonas* sp. , *Pseudoalteromonas* sp. , *Paracoccus* sp. (Lee *et al*., 2004) and *Vibrio* sp. (Kumar and Nair, 2007). These pigmented marine natural products also contribute to a variety of applications, from health, cosmetic up to the flavours of food additives, paint industry, fabric dye, and ink manufacture.. Active prodiginine derivatives have already entered clinical trials as potential drugs against different cancer types.

- **Carotenoids**

Several halophiles which were producing carotenoids isolated and characterized from solar salterns. (Pathak. and Sardar 2012) .

Arytenoids are main and most plentiful pigment group in marine pigmented bacteria which usually appears orange, yellow or red in colour.

Carotenoid pigment also exhibits antimicrobial including antibacterial activity (Gulani *et al*., 2012).

Haloarcula japonica, the extremely halophilic archaeon, produces carotenoids .

Carotenoids synthesized from *Haloarcula japonica*, have a function to protect their cells against the lethal actions of ionizing radiation, UV radiation, and hydrogen peroxide.

- **Melanin**

The ascomycetous black yeasts *Hortaea werneckii*, *Phaeotheca triangularis*, and *Trimmatostroma salinum* are halophilic fungi that inhabit hypersaline water of solar salterns. They are characterized by slow, meristematic growth and very thick, darkly pigmented cell walls. The dark pigment, generally thought to be melanin, is consistently present in their cell walls when they grow under saline and nonsaline conditions.(Kogej *et al* ., 2004) Melanin showed inhibitory activity against potential pathogens and activity was observed in *Salmonella typhi* and *Vibrio parahaemolyticus* . It was concluded that the melanin of *Hortaea werneckii* isolated from solar salterns possess a high antibacterial activity and could act as a suitable source of new antimicrobial natural products.(Rani *et al* ., 2013)

C) Antibiotics

Antibiotic is derived from antibiosis means “against life”. It can be purified from microbial fermentation and modified chemically or enzymatic ally for fundamental studies.

There are many strains of the genus *Bacillus* which can produce a wide variety of antibiotics including bacitracin, polymyxin, colistin etc. Several bacitracins have been characterized; bacitracin A is the dominant commercial product (Schallmey *et al*., 2004).

Bacillus antibiotics are generally produced at the early stages of sporulation. The transfer of the bacitracin biosynthetic gene cluster from *B. licheniformis* to the engineered host *B. subtilis* and the biosynthesis of bacitracin in high levels.

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.(Irshad *et al* ., 2013)

Berdy (2005) reported that *Actinomycetes* had antibacterial activity rather than anti fungal activity. In the group of antibiotics, 66% are antibacterial gram-positive and gram-negative, and 34% are anti fungi including yeast.

Soil and marine actinomycetes isolates can acts as potent source for novel antibacterial compounds against pathogenic bacteria.

Todkar . *et al* ., (2012) reported that halophiles present in marine environments are useful in control in diseases caused by bacterial and fungal pathogenic species. Protein extract from halophiles would have an increased importance in medicine and in health care industry again further research on the above aspects may be undertaken. Thus these protein purified extracts obtained from halophiles seems to be a potential source of arresting the growth and metabolite activities of various pathogenic microorganisms.

Antibiotic were produced by two strains *Bacillus pumilus* (NKCM 8905) and *Bacillus pumilus* (AB 11228) and tested against *E.coli*, *Staphylococcus aureus*, *Bacillus subtilis.* Maximum production of antibiotics from *Bacillus pumilus* against *Staphylococcus aureus* and *Bacillus subtilis* has been reported.(Sawale *et al* . , 2014)

Actinobacter strain Q70 had the ability of antibiotic production against methicillin resistant *Staphylococcus aureus*. Maximum antibiotic production was obtained in the medium containing 10% (w/v) NaCl. The strain Q70 had also activity against *Enterococcus faecalis*, *Bacillus subtilis* and *Pseudomonas aeruginosa.* .

(Tambekar *et al* ., 2014) reported that *Bacillus* strains were promising sources for the antimicrobial bioactive substances and represent a new and rich source of secondary metabolites that need to be explored.

Bacillus laterosporous was isolated from saline water. *Bacillus laterosporus* has shown significant effectiveness in eliminating Candida, improving and, in many cases, eliminating gastrointestinal symptoms and food sensitivities while enhancing the patient's digestive capacities.

Antimicrobial activity:- Foreshore soil of Daecheon beach and Saemangeum sea of Korea represents an untapped source of bacterial biodiversity . Most actinobacterial isolates are capable of antibacterial and antifungal metabolite production.

The antibacterial and antifungal profile of isolates from halophiles give the findings that these strains may contain multiple plasmids as seen in other plasmid containing strains. Some species of halobacteria have acidic proteins that resist the activity of most of other organisms. Maximum antimicrobial activity was observed against *E.coli* , *Pseudomonas aeruginosa*, *Bacillus subtilis* followed by *Klebsiella pneumonia.*

Microbacterium oxydans and *Streptomyces fradiae* showed anibactrial activity against all tested pathogenic bacteria and yeasts but not against pathogenic fungi.(Aarzoo *et al* ., 2013).

The halophiles isolated from Ratnagiri coastal area (marine environments) having antibacterial activity and they found to be gram negative non-motile organisms, shows the 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).

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