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Diversity of halophilic bacteria and actinobacteria from India and their biotechnological applications

Iti Gontia-Mishra*, Swapnil Sapre & Sharad Tiwari

Biotechnology Centre, Jawaharlal Nehru Agricultural University, Jabalpur 482004, India

*[E-mail: itigontia@gmail.com]

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This review summarizes the current diversity of halophilic bacteria and actinobacteria from Indian coastal region, salt lakes and their use in various biotechnological applications, including industrial, pharmaceutical, agricultural and environmental aspects. Culture dependent as well as culture independent methods for isolation of halophiles should go hand in hand to provide the insight mechanism of halophilic adaptation and their future applications.

[**Keywords**: Halophilic bacteria, Actinobacteria, Biodiversity, Biotechnological applications]

Introduction

Saline habitats are frequently inhabited by an abundance of microbial communities adapted to these ecosystems. Among the microorganisms, the bacteria play a major role as important and dominant inhabitants of saline and hypersaline environments[1]. Microorganisms that thrive in these environments have been broadly classified into halophilic microorganisms and halotolerant microorganisms. Halophiles are the microorganisms requiring salt for their growth whereas; halotolerant microorganisms are able to grow in the absence as well as in the presence of salt. Halophiles can be further divided into three categories according to their halotolerance, slight halophiles that grow optimally in 3% (w/v) total salt, moderate halophiles with optimal growth at 3-15% (w/v) salt and extreme halophiles that grow optimally at 25% (w/v) salt[2]. The world of halophilic microorganisms is highly diverse. Halophilic and halotolerant microorganisms are found in all three domains of life: Archaea, Bacteria and Eucarya. Actinobacteria, are filamentous Gram positive prokaryotes with 67-78% G + C content. Actinobacteria are considered as an intermediate group of bacteria and fungi and are recognized as prokaryotic organisms. Like bacteria, they are present in various ecological habitats and marine environments. Saline and hypersaline environments are found in wide variety of aquatic and terrestrial ecosystems. In terms of marine environment, India has coastline of about 8000 Km[3]. India is known for its rich biodiversity especially in context with halophiles because; it is surrounded by Arabian Sea in the west, Bay of Bengal in the east and Indian Ocean in the south. Besides this there are many salt lakes present in India such as Sambhar Lake, Rajasthan, Chilika Lake, Orissa etc. which are excellent source for isolation of halophiles. Marine environment is the prime reservoir of biological diversity and the marine microorganisms are recognized to be rich sources of novel compounds. Recently, there is accelerated interest in the study of marine halophiles and actinobacteria, with the aim of providing the information on microbial diversity and their role in biogeochemical cycling in marine ecosystems and in exploiting their ability to produce novel enzymes and industrially important bioactive substance like biosurfactants, extracellular polymeric substances (EPS), other crucial metabolites/compounds for biotechnological applications.

The present work summarizes the current diversity of halophilic bacteria and actinobacteria from Indian coastal region, salt lakes as well as other saline habitats and their use in various biotechnological applications, including industrial, pharmaceutical, agricultural and environmental aspects. Briefly, the mechanisms of adaption in saline environments and the molecular approaches to assess the diversity of halophiles will be discussed.

Diversity and habitat of halophiles

Halophiles have a diverse habitat. They are distributed in natural saline areas as well as in hypersaline environments. The natural saline environments vary from aquatic (e.g. salt marshes, salt lakes) to terrestrial (e.g. saline lands) [4,5]. Another important habitat for halophiles is artificial solar salterns used to mine salts from the sea, salt pans etc[6,7]. They are also present in association with the roots of halophytes and on surface of marine macroalgae[8,9]. Even, there are reports of halophiles in endosymbiotic association with trigger fish[10].

Bacteria characteristically include many kinds of halophilic and halotolerant microorganisms that are widespread in different phylogenetic subgroups. In Indian coastal region and hypersaline environments halophiles are reported from wide range of Gram positive rods and cocci e.g. *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* sp. and *Salinicoccus* sp.[11,12,13] and Gram negative bacteria e.g. *Halomonas* sp., *Vibrio* sp. and *Marinobacter* sp.[12,14,15]. Gram-negative and Gram positive bacteria have been frequently isolated from saline environments including saline water and saline soils. Actinomycetes/actinobacteria represents only a small fraction of the bacterial flora of saline soils[1]. There are reports of isolation of some halophilic actinomycetes such as *Actinopolyspora* sp., *Streptomyces* spp., *Micromonospora* sp. and *Saccharopolyspora* sp. from saline habitats of Indian coast regions[16,17,18]. There is ongoing interest in searching new bacterial and actinobacteria strains from various saline environments to study the mechanism of adaptation and enzymatic activity under saline conditions. The detailed habitats, isolation sites, isolate affiliation; NaCl tolerance and their application in diverse fields are shown in Table 1.

Mechanism of adaptation in saline environment

In order to adapt to the saline conditions, halophilic bacteria and actinobacteria have developed various strategies to their maintain cell structure and function[19]. There are two main strategies that halophiles have evolved to deal with high salt environments (i) "salt in" strategy and (ii) "compatible-solute" strategy. Bacterial cells maintain internal concentrations that are osmotically equivalent to their external environment. They maintain osmotically equivalent internal concentrations by accumulating high concentrations of KCl. For every three molecules of KCl accumulated, two ATP are hydrolyzed making this strategy more energy efficient than the "compatible solute" strategy. This mechanism is accompanied by certain physiological modifications which are required to protect all the metabolic and regulatory functions (e.g. enzymatic activity, synthesis of cellular components, and structure and function of some organelles) at high salinity[1]. The "salt in" strategy of osmoregulation is adopted by members from archaebacteria and eubacteria.

In the "compatible solute" strategy cells maintain low concentrations of salt in their cytoplasm by balancing osmotic potential with organic, compatible solutes. Compatible solutes include polyols such as glycerol, sugars and their derivatives e.g. trehalose, sucrose; amino acids and their derivatives e.g. proline, glutamate and quaternary amines such as glycine betaine. Compatible solutes could be synthesized *de novo* or, if present in the medium, can be taken up by the organisms[20]. Osmoprotectants are defined as exogenously provided organic solutes that enhance bacterial growth in media having high osmolarlity. These substances may themselves be compatible solutes, or they may act as precursor molecules that can be enzymatically converted into these compounds. Compatible solutes regulate cells by accumulation of them up to molar concentrations; compatible solutes lower the cytosolic osmotic potential and hence make major contributions to the restoration and maintenance of turgor[21]. Energetically this is an expensive process. This strategy of adaptation is followed by many halophilic eubacteria and actinobacteria. In addition to their well studied function as osmoprotectants, compatible solutes also have protein-stabilizing properties that support the correct folding of polypeptides under denaturing conditions both *in vitro* and *in vivo*[22].

Besides these strategies, bacteria have evolved some other possible mechanisms to adapt to saline environments by changing the composition of their cell envelope especially the exopolysaccharides. Sandhya et al.[23] reported that *Pseudomonas*, a halotolerant bacteria could survive under stress condition by producing exopolysaccharides, which protects them from fluctuations in water potential by increasing water retention and maintaining the diffusion of carbon sources in microbial environment. Similarly, *Halomonas variabilis* and *Planococcus rifietoensis* were reported to survive under salinity stress by exopolysaccharide production[24]. The Chemical composition of cell membranes is also occasionally modified and synthesis pattern of proteins, lipids, fatty acids and peptidoglycan are changed with a moderate increase in salinity. Various mechanisms of adaptation in saline conditions by bacteria and actinobacteria are summarized in Fig. 1.

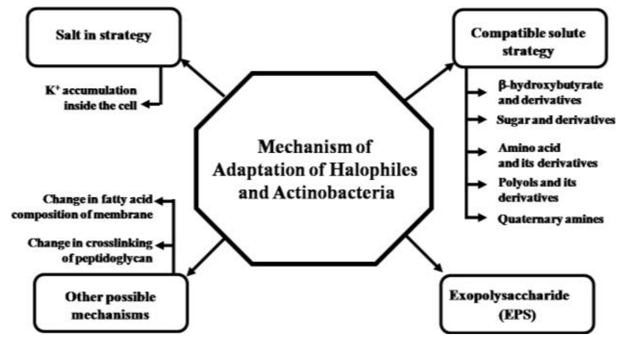

Fig. 1. Mechanisms of adaptation of halophiles and actinobacteria in saline environment

Table 1. Diversity of halophilic bacteria and actinobacteria from India

S.No.	Isolation sites	Source of isolation	Isolate affiliations	NaCl tolerance (w/v)	Applications	References
Bacteria						
1	Mithapur, Gujarat & Sambhar Salt Lake, Rajasthan	Marine salterns	*Halobacterium* MSW5, *Haloferax, Natronobacterium*	22.5%	-	4
2	Veraval Gujarat,	Soil	*Bacillus* sp.	20%	Protease	11
3	Solar saltern, Tuticorin, Tamilnadu,	Soil	*Halogeometricum borinquense*	20%	Protease, Serine protease	66,67
4	Saltpan of Port Okha, Gujarat	Soil	*Bacillus okhensis*	0-10%	Amylases, proteases, gelatinase	38
5	Lonar Lake Maharashtra	Sediment and water	*Halomonas campisalis*	23%	Lipase and polyhydroxyalkanoic acid (PHA) granules	35
6	Andaman and Nicobar Islands	Sea water	*Bacillus circulans*	3.5%	Biosurfactant	5
7	Solar saltern, Tamil Nadu	Water	*Haloferax lucentensis*	19.7%	Protease	6
8	Salterns and coastal regions of Kumta, Karnataka	Soil and water	*Bacillus aquimaris*	3%	Protease	68
9	Salt farm, Bhavnagar, Gujarat	Sediment	*Bacillus pumilus*	3-15%	Xylanase	7
10	Eastern regions of Uttar Pradesh	Soil	*Bacillus cereus*	0-10%	Protease	34
11	Chorao island of Mandovi estuary, Goa	Sediment	*Bacillus subtilis*	3%	Xylanase	42
12	Coastal region of Diu, India	Seawater	*Vibrio parahaemolyticus*	3.5%	Extracellular polymeric substances (EPS) production	46
13	Kovalam Saltpans in	Water	*Vibrio fischeri,Halobacillus salinus, Halobacterium*	4-25%	Amylases, proteases, gelatinase	12

	Kanyakumari, Tamil Nadu		*salinarum, Halococcus salifodinae, Bacillus subtilis, Natranobacterium* sp., *Staphylococcus epidermidis, Staphylococcus intermedius* and *Staphylococcus citreus*			
14	Veraval, Gujarat	*Gracilaria dura* (brown algae)	*Bacillus licheniformis*	3.5%	Extracellular polymeric substances (EPS) production	9
15	Kumta Coast Karnataka	Soil and water	*Halomonas hydrothermalis*	0-21%	-	69
16	Somnath Coast, Gujarat	Sea water	*Virgibacillus* sp.	3.5%	Protease	70
17	West coast of Karnataka	Soil	*Salinicoccus* sp.	2-25%	Amylase, Protease, Inulinase and Gelatinase	13
18	Salt Pan Ribander, Goa	Sea water	*Alkalibacillus* sp. *Virgibacillus panthotheticus*	15%	Protease	27
19	Salt pan, Khambhat, Gujarat	Soil	*Staphylococcus epidermidis, Bacillus* sp., *Halomonas shengliensis, Halomonas koreensis, and Virgibacillus*	5-15%	Bioremediation of metals (Cd)	60
20.	Coastal regions of Gujarat, Goa, Kerala and Sambhar Salt Lake, Rajashthan	Soil and water	*Marinobacter, Virgibacillus, Halobacillus, Geomicrobium, Chromohalobacter, Oceanobacillus, Bacillus* and Halomonas	3-20%	Amylases, lipases and proteases	14
21	Kutch, Gujarat	Sea water and soil	*Halomonas salina*	35%	Lipase	36
	Saltern pond of Tuticorin, Tamilnadu	Soil	*Chromohalobacter* sp.	15-25%	Xylanase	43
22	Chandigarh, Punjab	Soil	*Bacillus subtilis*	14%	Antimicrobial peptides	71
23	Kanyakumari, Tamil Nadu	Sea water	*Halomonas* sp.	2-20%	Biosurfactant	15
24	Vizhinjam Bay, Kerala	Trigger fish	*Halomonas aquamarina, Halomonas marina*	5-15%	-	10
25	Kanyakumari, Tamilnadu	Water	*Bacillus* sp.	5-20%	Biosurfactant, Antimicrobial, anticancerous metabolites	48
26	Salt pan, Bhavnagar, Gujarat	Soil	*Kocuria rosea*	0-20%	Bioremediation of dyes	61
27	Bhitarkanika coast of Orissa	Soil	*Bacillus* spp., *Microccocus luteus*	5-15%	Gelatinase, amylase	72
28	Kanyakumari, Tamil Nadu	Sea water	*Kocuria marina*	5-15%	Biosurfactant	50
29	Solar salterns of Mulund, Maharashtra	Sea water	*Halobacillus, Salicola, Halomonas, Pseudomonas, Haloferax*	0-20%	-	73
30	Solar salterns of Gujarat, Orissa, and West Bengal	Soil and water	*Halomonas, Salinicoccus,Bacillus, Aidingimonas, Alteromonas,* and *Chromohalobacter*	5-15%	Extracellular polymeric substances (EPS) production	47
31	Chilika lake, Odisha	Water	*Staphylococcus* sp.	5-15%	-	74
32	Parangipettai saltpan, Tamil Nadu	Sediment	*Bacillus* sp., *Halobacterium* sp, *Halobacillus* sp., *Halobacterium* sp., *Staphylococcus aureus, Halobacterium salinarum,*	3.5%	Antimicrobial metabolites	57

			and *Halobacillus salinus*			
33	Marakkanam saltpan Tamil Nadu	Sediment	*Bacillus* sp.	3.5%	Probiotics	54
34	Birbhum District, West Bengal	Soil	*Bacillus flexus*	20%	Bioremediation of florides	62
35	Muttukaadu, Chennai, Tamil Nadu	Sediment	*Bacillus subtilis*, *Bacillus endophyticus*	13%	Cellulase	44
36	Wayanad, Kerala	Soil samples	*Klebsiella pneumoniae*	-	Polyhydroxy-alkanoates production	91
37	Okha-Madhi, Gujarat	Salt enriched soil	*Oceanobacillus iheyensis* O.M.A18 and *Haloalkaliphilic bacterium* O.M.E12	-	Serine proteases	92
38	Kangra, Himachal Pradesh	Soil samples	*Halobacillus, Shewanella*, *Halomonas* and *Marinomonas*	3%		93
39	Bakreshwar, West Bengal,	Hot spring	*Bacillus cereus*	3–11%	Esterase production	94
40	Marakkanam, Tamil Nadu	Sediment samples	*Bacillus subtilis*		Amylase Production	95
41	Kannur, Kerala	Mangrove area	*Bacillus* sp. MG12	-	Biopolymer production	96
	Actinobacteria					
42	Alibag coast, Maharashtra	Marine sediment	*Actinopolyspora* sp.	10 to 15%	Antimicrobial metabolites	16
43	Saurashtra University Campus, Rajkot, Gujarat	Soil	*Streptomyces sannanensis*	5-7%	Antibiotic production	75
44	Mithapur, Gujarat	Soil	*Streptomyces clavuligerus*	0-10%	Protease production	33
45	West coast of Kerala	Soil	*Streptomyces* spp.	1-4%	Antimicrobial metabolites	55
46	Vellar estuary, Tamil Nadu	*Suaeda* salt marsh	*Actinopolyspora*	15-25%	Osmolyte production	8
47	Alibagh, Mumbai, Maharastra and Goa	Marine sediments	*Streptomyces* sp.	7%	Amylase	39
48	The Bay of Bengal coast of India	Marine sediments	*Saccharopolyspora salina*	1-26%	Antimicrobial metabolites	76
49	Marakkanam coast of Tamil Nadu	Sediments	*Streptomyces*	15%	Antimicrobial metabolites	77
50	Vellar Estuary, Tamil Nadu	Soil	*Streptomyces bikiniensis*	7%	Antimicrobial metabolites	78
51	Ennore saltpan, Tamil Nadu	Soil	*Streptomyces* sp.	3.5%	Antimicrobial metabolites, Chitinase, Biosurfactant	79,80
52	Okha, Gujarat	Salt enriched soil	*Nocardiopsis alba*	-	Alkaline protease production	97
53	Kanyakumari, Tamilnadu	Mangrove sediments	*Streptomyces* sp.	3.5%	Anticancer metabolites	56
54	Tamil Nadu	Marine water	*Streptomyces acrimycini*, *Streptomyces albogriseolus* and *Streptomyces variabilis*	3.5%	Bioremediation of Xenobiotics (Carbaryl) and heavy metals (Zn, Cu)	59
55	Tuticorin, Tamil Nadu	Soil	*Streptomyces* spp., *Nonomuraea* sp., and	4%	Antimicrobial metabolites	17

			Micromonospora sp.			
56	Bay of Bengal, Tamil Nadu	Marine sediment	*Streptomyces* sp.	3.5%	L-asparaginase production	81
57	Andaman and Nicobar	Sediment sample	*Saccharopolyspora, Streptomyces, Streptoverticillium, Nocardiopsis, Actinopolyspora, Microtetraspora*	5-30%	Biosurfactant, amylase, protease, cellulase and Antimicrobial metabolites	18
58	Ribandar saltern, Goa,	Sediment samples	*Streptomyces* spp, *Nocardiopsis, Micromonospora* and *Kocuria* spp.	32, 50, 75, 100%	Anti-bacterial metabolites production	98

Molecular approaches to assess the diversity of bacteria and actinobacteria

Molecular phylogeny is a very useful tool to analyze microbial populations. A number of molecular techniques are known for identifying and analyzing the biodiversity of bacterial strains, such as random amplified polymorphic DNA (RAPD), amplified ribosomal DNA restriction analysis (ARDRA), and 16S *rRNA* gene analysis. The comparison of 16S *rRNA* gene sequences is a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria[25]. Small-subunit of rDNA gene (16S rDNA) is widely used to establish phylogenetic relationship among bacteria because this gene is universally present, sufficiently small to be easily sequenced and a large database for it is available. Furthermore, rDNA that encodes *rRNA* comprises of highly conserved regions, crucial for structure and function, flanked by highly variable stretch, which varies among various species[26]. Identification and phylogenetic analysis of halophilic bacteria using 16S rDNA genes amplification has been extensively implemented by many authors[15,27]. 16S rDNA primers and probes for specific identification of actinomycetes and especially for streptomycetes were designed by Stackebrandt et al.[28] (1991) and Mehling et al.[29] (1995). ARDRA and 16S rRNA gene sequencing have been applied to characterize many actinobacteria[18,30]. Additionally, there are degenerate primers for genes encoding polyketide synthases (PKS-1 and PKS-2) and non ribosomal peptide synthetase (NRPS) which are used to screen the biosynthetic potential in terms of natural product drug discovery as identification of these genes provides indirect evidence of potential chemical diversity among the actinobacteria[31,32].

Applications of Halophiles

In the recent years, studies on halophilic microorganisms have significantly increased.

Halophiles directly or their products such as enzymes, antimicrobial compounds, exopolysaccharides, biosurfactants etc. finds vital application in diversified fields ranging from industries, pharmaceuticals, food industries, environment and agriculture (Fig. 2). The bacterial strains and their respective industrial, pharmaceutical and environmental applications are mentioned in Table 1.Industrial applications

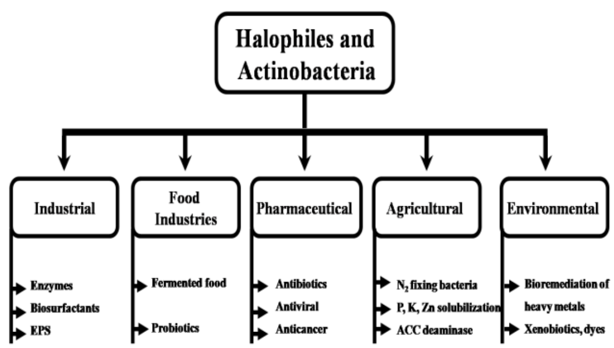

Fig. 2. Multifarious applications of halophiles and actinobacteria

Enzymes

Recently, the scientific interest has been shifted towards enzymes derived from halophilic bacteria due to their potential industrial applications. These enzymes are stable and actively function under extreme conditions. Halophilic bacteria and actinobacteria are known to produce diverse range of industrially important enzymes such as proteases, gelatinases, amylases, lipases, inulinase, cellulases etc.

Marine bacteria and actinobacteria are capable of producing enzymes with good stability at higher temperature and alkaline conditions.

Proteases: Halophilic proteases are widely used in the detergent and food industries. They have been also extensively employed in the baking, dairy and leather industries. Proteolytic activity with potential industrial applications has been characterized in many halophilic bacteria and actinobacteria from Indian coasts such as *Bacillus* sp., *Haloferax lucentensis*, *Bacillus cereus*, *Streptomyces clavuligerus*[6,11,33,34].

Lipase: Halophilic lipases are broadly used as detergent additives, in food and paper industries and as enantioselective biocatalysts for the production of optically pure chemicals. These enzymes have also been used as ingredients in laundry detergents for the removal of oil/grease stains. Lipolytic activity with prospective in industrial applications has been known from many halophilic bacteria from Indian coasts such as *Halomonas campisalis*, *Marinobacter* sp. and *Halomonas salina*[14,35,36].

Amylase: Amylases are widely employed in different biotechnological applications including the food industry in bread and baking industry to improve the volume of dough, colour and crumb softness. They are also applied in detergents to promote stain removal and in the paper and pulp industry for the modification of starches for coated paper and in the textile industry during the de-sizing process[37]. Amylase activity with definitive industrial applications has been known from many halophilic bacteria and actinomycetes from Indian coasts such as *Bacillus okhensis*, *Vibrio fischeri*, *Streptomyces* sp. and *Actinopolyspora*[12,18,38,39].

Xylanase: Xylanases play an important role in the degradation of xylan. They are used in the manufacture of coffee, livestock feeds and in baking industry to improve the properties of dough[40]. They can also be used in place of chlorine bleaching (biobleaching) for the removal of residual lignin from pulp. They are also employed in green energy production, assisting in the conversion of biomass to bioethanol and biodiesel[41]. Xylanase activity has been reported from only few halophilic bacteria from India such as *Bacillus pumilus*, *Bacillus subtilis* and *Chromohalobacter* sp.[7,42,43].

Cellulase: Cellulases are mainly applied in textile industry for biopolishing of fabrics and in laundry detergents for fabric softening and brightening, saccharification of agricultural and industrial wastes and in animal feeds[44]. They are also utilized in the production of bioethanol as the enzymes are used to hydrolyse pretreated cellulosic materials to fermentable sugars[45]. Currently, halophilic cellulases have been derived from *Bacillus subtilis*, *Bacillus endophyticus* and *Saccharopolyspora* from Indian coastal regions[18,44].

Exopolysaccharides (EPS)

Exopolysaccharides are biosynthetic polymers mainly consisting of carbohydrates secreted by bacteria. Bacterial extracellular polysaccharides have found different applications as gelling agents and emulsifiers in food industry, microbially enhanced oil recovery in petroleum industry, pharmaceutical and bioremediation agents in environment management system[9]. They are also used as adhesives in detergents, textiles, papers, paints and beverages industries. Several halophilic microorganisms produce such EPS in profuse amounts and therefore are commercially exploited. Several EPS producing halophiles have been reported from Indian coastal areas such as *Vibrio parahaemolyticus*, *Bacillus licheniformis*, *Salinicoccus* sp. and *Chromohalobacter* sp.[46,47]. Similarly, poly-β-hydroxyalkanoate (PHA), a polymer containing β-hydroxybutyrate and β-hydroxyvalerate units, is accumulated by many prokaryotes. It is used for the production of biodegradable plastics. Some halophilic bacteria of Indian origin also produce PHA e.g. *Halomonas campisalis*[35].

Biosurfactant

Biosurfactants are biological surface-active compounds released by microorganisms that have some influence on interfaces[48]. They are amphiphilic compounds produced on microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic moieties. The wide range of structural diversity in biosurfactants, results in a broad spectrum of potential industrial applications including production of food, cosmetics and pharmaceuticals, agriculture, herbicide and pesticide formulations, detergents, pulp and paper. They also find important application in mining, enhanced oil recovery, transportation of crude oil and soil remediation[49]. There are reports of biosurfactant production by many halophilic bacteria/actinobacteria such as *Bacillus circulans*, *Kocuria marina Halomonas* sp.[5,14,50].

Food industry

Various halophilic enzymes such as protease, amylase, xylanase and lipase have been extensively utilized in different step of food processing which have been discussed in section 5.1.1. Other applications of halophiles in food industry are mention below.

Fermented food

The industrial production of fermented products, like soy sauce and fish sauce that uses the degradative properties of halophiles. The fermentation of salty foods such as Chinese fermented beans, salted cod, salted anchovies, sauerkraut, often involves halobacteria as essential ingredients. Halophiles including *Halobacteria*, *Halococci* and *Natronococci* have been isolated from various food sources including fermented foods and sauces, including kimchi and Thai fish sauce[51]. A culture independent method of isolation of bacteria diversity from kimchi showed the presence of many halophilic bacteria including lactic acid bacteria[52].

Probiotics

Probiotics are live microorganisms thought to be beneficial to the host organism. These are commonly consumed as part of fermented foods with specially added active live cultures. Recently, halophilic lactic acid bacteria *Tetragenococcus halophilus* was isolated from soy sauce and showed to possess an immunomodulatory activity that promotes T helper type 1 immunity in humans. Thus this strain can be efficiently used as probiotics for humans[53]. Similarly, the use of halophilic *Bacillus* sp. as probiotics for shrimps has been reported from India[54].

Pharmaceutical applications

The mechanism of drug resistance among the pathogenic microorganisms lessens the efficacy of available antibiotics and this in turn strengthens the need to search new antibiotics. Marine microbes are continuously explored for production of novel antimicrobial compounds. Bioactive compounds from halophilic bacteria and actinobacteria have typical features because of their varied environmental conditions (pH, temperature, salinity, pressure, etc). Among marine microorganisms, actionbacteria are recognized as most promising prokaryote for novel bioactive metabolite production. There are several reports of production of antimicrobial metabolites, antimicrobial biosurfactants and anticancer agents by halophilic bacteria and actinobacteria such as *Staphylococcus aureus*, *Bacillus* sp., *Streptomyces* spp.[48,55,56,57].

Environmental applications

The large numbers of contamination sites are often saline to hypersaline and halophiles are prevalent in such environments making their significant utilization in bioremediation of contaminants. The accelerated industrial activities such as mining and metal plating resulting in pollution, due to the release of the high amount of organic and heavy metals into the environments. These toxic compounds are often found in runways and accumulate near seashores. Due to the evaporitic nature of hypersaline environments, heavy metals are frequently found in concentrated brine. As a result, many halophiles have developed tolerance to heavy metals[58]. There are some reports of utilization of halophiles for bioremediation of xenobiotic compound like carbaryl, a potent insecticide and heavy metals like Zn and Cu by actinobacteria like *Streptomyces acrimycini*, *Streptomyces albogriseolus* and *Streptomyces variabilis*[59]. In addition, the use of halophilic bacteria and actinobacteria for bioremediation of Cd has been reported by Solanki and Kothari[60]. The textile industry produces a large quantity of polluted wastewater containing azo dyes, phenol and other toxic anions. These effluents are highly saline with typical salt concentrations of 15-20%. Recently, a halophlic bacterium *Kocuria rosea* has been reported to decolorize triphenyl methane dyes like malachite green, crystal violet and methyl violet[61]. Similarly, fluorides are prevalent in environment and have cytotoxic effect on humans. A halophilic bacteria *Bacillus flexus* has been reported to reduce fluoride concentration up to 67.45% in contaminated soil[62]. These halophilic microbes play a bioremediative role by transforming these anions and xenobiotics into less toxic forms.

Agricultural applications

Soil salinity is a naturally occurring problem in various parts of the world, but the exhaustive use of chemical fertilizers, inadequate cultivation practices, and improper irrigation schemes management have resulted in exacerbated salt concentrations in soil. Salinity is one of the important abiotic stresses that limit the plant growth and crop productivity. In addition, salinity also affects nutrient uptake by plants. Agriculture under saline conditions already presents major challenges in many countries. Application of halotolerant plant growth promoting rhizobacteria (PGPR) is an important strategy by which cultivation in saline soils can be improved[63]. Many reports have been published stating the beneficial effects of inoculation of halotolerant bacteria on plant growth under salt stress conditions such as *Micrococcus* sp. on cowpea, *Brachybacterium saurashtrense* on groundnut etc[64,65]. Few of the halotolerant bacterial strains isolated from India and their plant growth promotion effect on respective plants are mentioned in Table 2.

Table 2. Halotolerant bacteria and their role in plant growth promotion under saline conditions

S. No.	Source of isolation	Isolate affiliations	NaCl tolerance	Plant growth promoting activity	Interaction with plant	Reference
1	Roots of mangrove-associated wild rice, Tamil Nadu	*Swaminathania salitolerans*	3%	N_2 fixation and phosphate solubilizing	-	82
2	Roots of mangrove plants Pichavaram, India	*Azotobacter chroococcum*, *A. virelandii* and *A. beijerinckii*	3%	N_2 fixation, IAA production	*Rhizophora*	83
3.	Rhizosphere soil samples from coastal ecosystems of Tamil Nadu	*Pseudomonas fluorescens*	0.7%	ACC- deaminase activity	*Arachis hypogaea*	84
4.	Rhizospheric soil of wheat from Varanasi, Mau, Ballia, and Ghazipur of Uttar Pradesh	*Bacillus* sp., *Arthrobacter* sp.	0-8%	Phosphate solubilization, IAA and Gibberellin production, siderophore,	-	85
5	Root-free soil, west coast of India	*Micrococcus* sp.	7%	Phosphate solubilization, IAA production, ACC-deaminase activity, siderophore	Cowpea	64
6	Coastal areas of Gujarat	*Pseudomonas fluorescens, P. aeruginosa, P. stutzeri*	6%	Phosphate solubilization, IAA production, ACC deaminase activity, siderophore	Tomato	86
7	Roots of *Salicornia brachiata*, coastal area of Gujarat	*Agrobacterium tumefaciens, Zhinguelliuella somnathii, Vibrio, Brachybacterium saurashtrense, Brevibacterium casei, Haererohalobacter* sp.	1-15%	N_2 fixation, phosphate solubilization, IAA production, ACC-deaminase activity, siderophore	*Salicornia, Arachis hypogaea*	63, 87, 65, 99
8	Rhizospheric soil of wheat from Mau, Ghazipur , Ballia, U.P. and Sambhar salt lake, Rajasthan	*Bacillus pumilus, Pseudomonas mendocina, Arthrobacter* sp., *Halomonas* sp., and *Nitrinicola lacisaponensis*	0-22%	Phosphate solubilization, IAA production, siderophore, and ammonia production	Wheat	88
9	Soil sample Jaisalmer, water from Sambhar and Pushkar lake, Rajasthan	*Bacillus* sp. and *Hallobacillus* sp.	5-25%	phosphate solubilization, IAA production, siderophore	Wheat	89
10	Rhizopheric soil of Grass, Rae Bareilly, Uttar Pradesh	*Bacillus pumilus, Halomonas desiderata, Exiguobacterium oxidotolerans*	2.5%	Phosphate solubilization, IAA production, ACC-deaminase activity, siderophore	*Mentha arvensis*	90
11	Rhizospheric soil of *Sorghum bicolor* Rajasthan, India	*Klebsiella* sp.	0-10%	Phosphate solubilization, IAA production, ACC-deaminase activity	Wheat	100

Conclusion

In the current scenario, both academic and industrial research mainly focuses on marine microorganisms due to its impulsive potential. The importance of halophilic bacteria and actinobacteria as potential applications has been recognized in various fields. This review presents the current status of research on the biology of halophilic bacteria and actinobacteria from Indian coastal areas and salt lakes. The diversity and distribution of these bacteria and actinobacteria are quite interesting ranging from different ecosystems such soil, water, association with plants, macroalgae and animals. These diverse ecosystems are potentially very useful sources for novel enzymes, metabolites with unique properties and have great biotechnological potential. These halophilic microbes from Indian coastal areas have extensive applications in industries, environment and agriculture. Still continuous efforts must be made to isolate new halophiles with efficient osmolyte production and other applications such as bioremediation of heavy oils and metals. Research efforts are necessary in order to estimate the prospective of these microorganisms to be applied in industrial, pharmaceutical and environmental processes.

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