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Halophilic Actinomycetes: Salt-loving Filaments

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ABSTRACT:
A large number of halophilic organisms belonging to the group actinomycetes have been isolated and characterized from various locations around the world in recent times. A few have been reported from Indian soils as well. Certain species have been studied for their tolerance to organic solvents and metal ions, and also production of extracelluar enzymes in presence of solvents and/or metals. Potential applications of these organisms in bioremediation and industry have been elucidated. Such organisms may also serve as good model for studies on stress response among bacteria.

Key words: Actinomycetes, Bioremediation, Halophiles, Metal tolerance, Solvent tolerance

INTRODUCTION

Actinomycetes are a heterogeneous group of gram-positive bacteria with a high G+C content. They produce branching mycelium of two types- substrate and aerial. Actinomycetes are found in diverse habitats (marine, soil, saltern, mangroves) and diverse environmental condition (covering a wide range of pH, temperature, salinity, heavy metals, etc.). Quite a few of them are capable of growing in salt-rich habitats, and are thus halophilic or halotolerant (Box 1).

Box 1. Glossary

- **Actinomycete**: A group of filamentous gram-positive, aerobic bacteria with a high G+C content.
- **Halophile**: An organism requiring salt (NaCl) for growth.
- **Halotolerant:** Capable of growing in presence of NaCl, but not requiring it.
- **Heavy metals**: Chemical elements with high atomic weight, and specific gravity at least 5 times that of water. This group of metals include transition metals, lanthanides, metalloids, and actinides.

Halophiles have been classified by Kushner [1] on the basis of their salt requirement into 5 categories (Table 1). Strains of halophilic actinomycetes having the potential of secreting extracellular enzymes (protease, lipase, esterase, galactosidase, amylases, etc.), which work well in alkaline pH range, tolerating high concentrations of organic solvents in their environment have been reported in last few years [2-4]. Their ability of producing antibiotics has also been explored [5]. New species of halophilic actinomycetes have been isolated from saline soils of different locations (Table 2). Halophilic actinomycetes have also been isolated from sea anemone [6]. Some of the genera whose members include halophilic actinomyctes are listed below [11]:

Table 1. Classification of halophiles based on salt requirement [1]

Category	Optimum salt requirement	Representative actinomycete
Non halophilic	< 0.2 M	*Saccharopolyspora gloriosae* [7]
Slight halophilic	0.2-0.5 M	*Demequina aestuarii* [8]
Moderate halophilic	0.5-2.5 M	*Saccharomonospora saliphila* [9]
Borderline extreme halophilic	1.5-4.0 M	*Streptomyces tritolerans* [10]
Extreme halophilic	2.5-5.2 M	*Actinopolyspora spp*. [5]

Nesterenkonia: This genus of the family *Micrococcaceae* was first proposed by Stackebrandt et. al. in 1995 and later amended by Collins et. al. (2002), and Li et. al. (2005). Currently this genus has eleven species, out of them six are halophilic, with three being actinomycetes [12].

Prauserella: This genus proposed by Kim and Goodfellow (1999) has been named after Helmut Prauser, a German microbiologist who made notable contributions to actinomycete systematics. Seven of its species are halophilic actinomycete. *Prauserella halophila* and *Prauserella alba* are relatively novel examples.

Georgenia: This genus from family *Bogoriellaceae* was proposed by Altenburger et. al. (2002), and later amended by Li et. al. (2007). Currently it contains three species, among whom one (*G. halophila*) is a moderately halophilic actinomycete.

Isoptericola: It belongs to the family Promicromonosporaceae, and was proposed by Stackebrandt et. al. (2004). Among four members of this genus, one (*I. halotolerans*) is halophilic actinomycete.

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Table 2. Halophilic actinomycetes discovered between 2000-2010

Name of organism	Isolated from	Reference(s)
Haloactinopolyspora alba	Salt lake, Xinjiang province, North-west China	[13]
Haloactinobacterium album		[14]
Georgenia halophila		[15]
Amycolatopsis halophila		[16]
Prauserella salsuginis, P. flava, P. aidigensis, P. sediminis, P. alba, P. halophila		[17,18]
Saccharopolyspora qujiaojingensis		[19]
Saccharopolyspora halophila		[20]
Streptomonospora amylolytica *Streptomonospora flavalba*		[21]
Nesterenkonia halophila		[22]
Nesterenkonia halotolerans		[23]
Nesterenkonia xinjiangensis		
Streptomonospora alba		[24]
Streptomonospora halophila		[25]
Haloactinospora alba		[26]
Nocardiopsis salina		[27]
Saccharomonospora paurometabolica		[28]
Nocardiopsis xinjiangensis		[29]
Nocardiopsis rosea		[30]
Nocardiopsis gilva		
Nocardiopsis rhodophaea		
Nocardiopsis chromatogenes		
Nocardiopsis baichengensis		
Haloglycomyces albus		[31]
Isoptericola halotolerans	Saline soil, Qinghai province, North- west China.	[32]
Nocardiopsis litoralis	Sea anemone collected from South China Sea, China	[6]
Nesterenkonia jeotgali	Jeotgal, a traditional Korean fermented seafood	[33]
Nocardiopsis kunsanensis	Saltern in Kunsan, Republic of Korea.	[34]
Nocardiopsis terrae	Saline soil, Qaidam basin, North-west China	[35]
Streptomyces tritolerans	Soil from Gulbarga Karnataka, India	[10]
Saccharomonospora saliphila		[9]
Actinopolyspora species AH1	Marine sediments sample from Alibag coast Maharashtra, India	[5]
Streptomyces clavuligerus	Mithapur, west coast of India	[36]
Nocardiopsis prasina HA-4	Limestone quarry, Ukhrul district, Manipur, India	[4]
Actinopolyspora spp.	Oil field, Sultanate of Oman	[37]
Demequina aestuarii	Tidal flat sediment sample, South Korea	[8]
Nocardiopsis halotolerans	Marsh soil, Kuwait	[38]
Saccharomonospora halophila		[39]
Nesterenkonia aethiopica	Abjata soda lake, Ethiopia	[40]
Streptomyces spp.	Saltpan soil	[41]

Haloactinobacterium: This genus of family *Ruaniaceae* was proposed by Tang et. al. (2010), and has only one member characteized as halophilic actinomycete.

Amycolatopsis: This genus was proposed by Lechevalier et. al. (1986). Out of the forty-one species in it, just one is actinomycete.

Saccharomonospora: It belongs to the family *Pseudonocardiaceae* and was proposed by Nonomura and Ohara (1971). It comprises of eight species, from which three are actinomycetes.

Saccharopolyspora: This genus was proposed by Lacey et al (1975). It comprises of twenty species, from which two are actinomycetes.

Haloactinospora: It is a newly formed genus with only 1 representative member *Haloactinospora alba*.

Nocardiopsis: It belongs to the family *Nocardiopsaceae*, which was proposed by Rainey et al in 1996 [1]. This particular genus was proposed by Meyer (1976) [29]. Currently it comprises of 38 species including 11 actinomycetes.

#Box 2. List of media used for isolation of halophilic actinomycetes.

Bennet media [33, 36]
Cellulose casein multi salts media [25]
Chitin agar [36]
Czapek agar [17, 23, 24, 28]
Glucose aspargine agar [27, 28]
Glycerol aspargine agar(ISP5) [22-24, 28, 29]
Inorganic salt starch agar(ISP4) [24, 23, 28]
Maltose yeast extract agar [24, 28]
Marine agar [16, 22, 32] / Marine broth[33]
Modified Horikoshi medium [31]
Medium glycerol glycine MSG media [21]
Nutrient agar [23, 24, 28]
Oatmeal agar(ISP 3) [24, 28]
Potato extract [23,24,28, 29]
Salt starch nitrate agar [38, 39]
Skim milk agar [36]
Starch casein agar [17,23,41]
Starch casein nitrate agar [36]
Trypticase soy broth [32, 33]

#Appropriate amount of salts are added in media as per growth requirement of organism(s) in question.

Streptomonospora: This genus of family *Nocardiopsaceae* was proposed by Cui et. al. (2001) [24]. It comprises of 5 representatives of halophilic actinomycetes. Streptomonospora is a group of extremely halophilic filamentous actinomycetes. Members of which are isolated frequently, probably due to the high occurrence of these actinomycetes in the hypersaline soil environment.

ISOLATION AND CULTIVATION

While attempting isolation, sampling is an important aspect demanding considerable attention. After collection, the samples should be examined immediately or aseptically stored under refrigeration. A variety of media are known for the isolation and characterization of different halophilic actinomycetes (Box 2). Growth media may be formulated according to the ecosystem or habitat from which sampling is done, e.g. seawater can be added to isolation agars at different concentrations to match the salt gradient in an estuary. Antifungal agents are usually incorporated into isolation agars to retard the fungal development.

APPLIED ASPECTS

Bioremediation

Heavy metal tolerance

Heavy metals are being introduced into the water bodies by various industries. Heavy metals such as lead, cadmium, copper, zinc, mercury, arsenic, and chromium in effluents from tanneries and fertilizer industries are released into nearby streams and rivers. Many heavy metals are released as by-products of different industries like leather industries, sugar mills, textiles, and fertilizer industries. Heavy metal contamination of the water bodies affects the flora of water bodies, thus disturbing the ecosystem. It can also cause health problems to animals and human beings [42]. Sponges filter large volumes of seawater and potentially accumulate heavy metals and other contaminants from the environment into their tissues. One study concerning the heavy metal resistance of sponge associated bacteria was reported to develop suitable biological indicator [43].

There are few reports on interaction between moderate halophiles and heavy metals [44, 45]. Bacteria tolerating heavy metals have been studied for the mechanism of tolerance. Some are responsible for environmental transformation and thus can be used as bioassay indicator in saline/non-saline, polluted and non-polluted environments [46]. Salinity of the medium may have an impact on metal tolerance of the organism [47]. Hypersaline soil and water often get polluted with heavy metals and other toxic material. During the manufacture of chemicals such as herbicides, pharmaceuticals, pesticides and during oil and gas recovery processes hypersaline waste water is generated. Normal remediation treatment may not work at such high salt concentration, so here halophilic organisms that work well at high salt concentrations can prove a useful alternative [48].

Some halophilic actinomycetes (*Streptomyces sp*.) were evaluated for their efficiency to bioremediate heavy metals. Two strains VITDDK1 and VITDDK2 isolated from Ennore saltpan showed significant heavy metal resistance. Both isolates grew well in the presence of zinc sulphate, cadmium, lead acetate, and cadmium sulphate [49]. Acidophilic and chemolithotrophic microorganisms are widely used for bioleaching purposes. Several alkaliphilic microorganisms also have bioleaching capacity. One such organism which prefers light, alkaline conditions (pH 8.5) for growth is *Nocardiopsis metallicus*. It is a halophilic actinomycete isolated from Germany. Due to its ability to solubilise metals, it may prove useful in mobilizing metals from soils, solid waste materials, and ores [50].

Crude oil degradation

Oil spillage is one of the main causes of environmental pollution. In a study involving multiple cultures of alkaliphilic, halophilic, and thermophilic actinomycetes, a thermophilic actinomycete *Thermoactinomyces dichotomicus* 84TH, isolated from soil in Georgia showed a high efficiency of oil hydrocarbon detoxification [51]. *Rhodococcus sp*. NCIM 5126, a marine actinomycete isolated from Bombay harbour could degrade aliphatic and aromatic fraction of crude oil, so it can be useful for bioremediation of soil and aquatic systems contaminated by hydrocarbons [52].

Enzymes

Biocatalysts offer advantages over the use of conventional chemical catalysts for numerous reasons, for example they exhibit high catalytic activity, a high degree of substrate specificity, can be produced in large amounts and are economically viable.

Alkaline proteases are produced by different bacteria and fungi, but less study is being done on production of alkaline proteases by alkaliphilic actinomycetes. Proteases are very important industrial enzymes which constitute 60% of global enzymes sales. They constitute two thirds of the total enzymes used in various industries and it account for at least a quarter of the total global enzyme production [53-54]. Few reports describe extracellular alkaline proteases produced by certain halophilic and alkaliphilic bacteria [55]. *Nocardiopsis prasina* HA-4, isolated from Manipur (India) produced protease, and could tolerate a wide range of temperature (20-42^{0}C) and pH (7-10). The optimum temperature for the enzyme was 55°C and it had two pH optima at 7 and 10. Effect of certain metal ions (Ca^{2+}, Fe^{2+}, Mg^{2+}, Hg^{2+}) was also studied on enzyme production. Fe^{2+} (as $FeSO_4$) had stimulatory effect on HA4 enzyme activity, Ca^{2+} and Mg^{2+} had negative effect, whereas Hg^{2+} completely inhibited HA4 protease. Enhancement of protease activity by Fe^{2+} has been rarely reported [4]. Another salt tolerant alkaliphilic actinomycete *Streptomyces clavuligerus* strain Mit-1 isolated from Mithapur, western coast of India has also been reported to produce alkaline protease [36], and so as the alkaliphilic actinomycetes isolated from marine sediments of the Izmir Gulf, Turkey, strain MA1-1 [2].

Nocardiopsis halotolerans isolated from salt marsh in Kuwait was found to be slightly keratinolytic. It grew at 28–35^{o}C in salt concentrations of 0–15% [38]. *Saccharomonospora halophila*, another isolate from Kuwait, utilized keratin as sole carbon and nitrogen source [39].

One lignin oxidizing enzyme, lignin peroxidase (ligninase) is produced by *Streptomyces psammoticus*. Lignin is the most complex biopolymer and it plays a major role in carbon cycle. Lignin degrading enzymes have industrial applications such as textile dye decolourization, delignification of pulp and effluent treatment [56].

Biosurfactants

Chemical surfactants are surface active agents that reduce the surface tension, and are mostly petroleum derivatives. They are highly toxic and non-degradable in nature. Bio-surfactants are surface active agents produced by certain microorganisms. They are amphiphilic, non toxic, eco-friendly, with high foaming ability, and biodegradable in nature [57, 58]. They are stable at extreme conditions like high temperature, high salt concentration, etc. Bio-surfactants can thus be used as an alternative for chemical surfactants [49]. Marine actinomycetes are good candidates for bio- surfactant production [59]. *Streptomyces orientalis* and *S. aureomonopodiales* exhibited good biosurfactant activity at high temperature, pH and salt concentration [49].

Solvent tolerance

Solvent tolerance is a strain-specific property, and the toxicity of a given solvent correlates with the logarithm of its partition coeficient in n-octanol and water (log P_{ow}). Organic solvents with a log P_{ow} between 1.5 and 4 are extremely toxic for microorganisms and other living cells because they partition preferentially in the cytoplasmic membrane, disorganizing its structure and impairing vital functions [36], changing structural and functional integrity followed by cell lysis [60]. In presence of organic solvents, normally enzymes are easily denatured and their catalytic activities deminish [61]. Thus enzymes which naturally remain stable in the presence of solvents could be very helpful for biotechnological applications in which these types of solvents are used [62]. Halophilic extremozymes can keep their catalytic properties in organic solvents, even at low salt concentrations. The use of these extremozymes in organic media could open new fields of application [63].

The halophilic α-amylase isolated from *Nesterenkonia sp.* strain F from Aran-Bidgol lake (Iran) could tolerate various organic solvents such as chloroform, cyclohexane, benzene, and toluene. Such properties of halophilic α-amylases could be exploited in starch-processing industries, baking, brewing, textile, and distilling industries [64]. A salt-tolerant alkaliphilic actinomycete, *Streptomyces clavuligerus* isolated from Mithapur, coastal region of Gujarat, India could grow up to 15% salt and pH 11, the optimum being 5% salt and pH 9. It was able to tolerate, and secrete alkaline protease in the presence of xylene, ethanol, acetone, butanol, benzene, and chloroform (each solvent upto 2%v/v). The solvents were tested with crude, partially purified and purified enzyme. The enzyme secretion was increased by 50-fold in presence of butanol. It could also utilize these solvents as the sole source of carbon with significant enzyme production [36].

FINAL COMMENTS

Several halophilic actinomycetes have been studied for solvent tolerance, and metal tolerance, and how presence of solvents or metals affect extracellular enzyme production in them. It will be interestingly useful to decipher the strategy by which they tolerate such multiple stresses (high salt, metal, solvent) and still maintain catalytic efficiency of their enzymes. Whether and how these enzymes differ from those produced by other organisms needs to be explored, which may lead to significant novel biotechnological applications. Additionally such hardy organisms which are simultaneously withstanding high salt, multiple metal ions or organic solvents, can serve as good model for investigating stress response in prokaryotes.

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