

Review

## Halophilic microbes for bio-remediation of salt affected soils

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**Bacteria inhabiting soil play a role in conservation and restoration biology of higher organisms. The salt affected soils are dominated by many types of halophilic and halotolerant microorganisms, spread over a large number of phylogenetic groups. These microbes have potential for plant growth promotion and release of enzymes under salt stress. Halophiles have thus potential to remediate salt affected soils, enhancing plant growth and yield under high salt containing soils where plant growth is restricted. This eco-friendly approach for bio-remediation of salt affected soils to optimize crop yields under stress through halophiles has gained importance among researchers in recent past.**

**Key words:** Halophiles, bio-remediation, saline soils, salt tolerant bacteria.

### INTRODUCTION

Life exists over the whole range of salt concentrations encountered in natural habitats: from freshwater environments to hypersaline lakes or seas, and other places saturated with respect to sodium chloride especially saline and saline-alkali soils. In many cases, the soil properties are the most defintory of the limitations for the ecosystem functioning and, quite always, the soil is the component of the ecosystem more resilient to changes. The influence of the high salt concentrations masks other soil forming processes or soil properties and environmental conditions, often altering them.

The soil that contains excess salts which impairs its

productivity is called salt-affected (Figure 1) (Chhabra, 1996). Salt accumulation in soil is characterized by saline soil, contains high amount of soluble salts  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$  and  $\text{Na}^+$  salt of  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$  and  $\text{CO}_3^{3-}$  etc; Sodic soil, dominated by  $\text{Na}^+$  salt and saline-sodic soil that have high salt of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  as well as  $\text{Na}^+$ . Salt-affected soils occupy an estimated 952.2 million ha of land in the world which constitutes nearly 7% of the total land area and nearly 33% of the area of potential arable land. In India, the salt affected soils account for 6.727 million ha (2.1%) of geographical area of the country. A build-up of soluble salts in the soil may influence its behaviour for crop production through changes in the

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**Figure 1.** Salt affected soils.

proportions of exchangeable cations, soil reaction, physical properties and the effects of osmotic and specific ion toxicity.

Physical and chemical methods of their reclamation are not cost-effective and also the availability of gypsum or other chemical amendments is a problem. The applications of halophilic bacteria include recovery of salt affected soils by directly supporting the growth of vegetation thus indirectly increasing crop yields in salt affected soils. All halophilic microorganisms contain potent transport mechanisms, generally based on  $\text{Na}^+/\text{H}^+$  antiporters, to expel sodium ions from the interior of the cell (Oren, 2002). Also, some halophiles express ACC deaminase activity that removes stress, ethylene from the rhizosphere and some produce auxins that promote root growth. Halophilic microbes are also found to remove salt from saline soils (Bhuva et al., 2013). There are reports that potential salt tolerant bacteria isolated from soil or plant tissues and having plant growth promotion trait, helps to alleviate salt stress by promoting seedling growth and increased biomass of crop plants grown under salinity stress (Ravikumar et al., 2007; Chakraborty et al., 2011).

Endophytic salt tolerant bacteria residing within plant tissues have been reported to be promoting the plant growth directly or indirectly through production of phytohormones, bio-control of host plant diseases and improvement of plant nutritional status (Pandey et al., 2008; Rosenblueth and Romero, 2006; Arora et al., 2014). They also possess the capacity to solubilize phosphates (Hung and Annapurna, 2004; Son et al., 2006).

Plant-microbe interaction is beneficial association between plants and microorganisms and also a more efficient method used for the reclamation of salt affected soils. Bacteria are the most commonly used microbes in this technique. However, archaea, fungi, actinomycetes, etc that may be found in environments with high salt concentrations can also be effective. Thus, there is high potential for bio-remediation applications for salt affected soils using halophilic microbes.

### **Halophilic microbes**

The existence of high osmotic pressure, ion toxicity, unfavourable soil physical conditions and/or soil flooding, are serious constraints to many organisms and therefore salt-affected ecosystems are specialised ecotones. The organisms found there have developed mechanisms to survive in such adverse media, and many endemisms. The halophilic microorganisms or "salt-loving" microorganisms live in environments with high salt concentration that would kill most other microbes. Halotolerant and halophilic microorganisms can grow in hypersaline environments, but only halophiles specifically require at least 0.2 molar (M) of salt for their growth. Halotolerant microorganisms can only tolerate media containing  $<0.2$  M of salt. Distinctions between different kinds of halophilic microorganisms are made on the basis of their level of salt requirement and salt tolerance. According to Kushner (1993) classification, microbes responsive to salt are defined under five groups:

1. Non-halophilic,  $<0.2$  M (~1%) salt
2. Slight halophiles, 0.2-0.5 M (~1-3%) salt
3. Moderate halophiles, 0.5-2.5 M (~3-15%) salt
4. Borderline extreme halophiles, 1.5-4.0 M (~9-23%) salt
5. Extreme halophiles, 2.5-5.2 M (~15-32%) salt.

The halotolerant grow best in media containing  $<0.2$  M (~1%) salt and also can tolerate high salt concentrations. This definition is widely referred to in many reports (Arahal and Ventosa, 2002; Ventosa et al., 1998; Yoon et al., 2003).

### **Soil microbial diversity**

Microbial community in the soil are not distributed at random. Factors such as soil composition, organic matter, pH, water and oxygen availability, along with the

host plant, play major role in the selection of the natural flora (Ross et al., 2000). The soil gains importance, especially in saline agricultural soils, where high salinity results from irrigation practices and application of chemical fertilizer. This effect is always more pronounced in the rhizosphere as a result of increased water uptake by the plants due to transpiration. Hence, the rhizobacteria form a group of the best adapted microorganisms (Tripathi et al., 1998).

Saline or hypersaline soils have yielded many Gram-positive species, and these have been characterized taxonomically. The microbiota of hypersaline soils is more similar to those of nonsaline soils than to the microbiota from hypersaline waters. This suggests that general features of the environments are more important in determining the microbiota in a particular habitat than are individual factors such as high salinity (Quesada et al., 1983).

The structure and function of plant root system contributes to the establishment of the rhizosphere microbial population (Russell, 1977) and rhizosphere microbial communities are mainly determined by plant species (Marshner et al., 2001; Miethling et al., 2000) and soil characteristics (Degens et al., 2000). The microbial community found around the roots of plants or rhizosphere is generally much greater than in the surrounding soil environment (Nihorimbere et al., 2011). It reflects that the rhizosphere supports higher microbial growth rates and activities as compared to the bulk soil. One of the main reasons for these higher growth rates is the increased availability of soluble organic compounds that are the results of plant root exudation. These are typically carbohydrate monomers, amino acids and sugars, but the composition and quality of root exudates varies, depending on plant species and abiotic conditions such as water content and temperature. The organisms most commonly found in environments with high salt concentrations are bacteria, archaea, fungi, viruses, actinomycetes and vesicular arbuscular mycorrhiza (VAM).

Industrial biocatalysis is found in the halophilic microorganisms, a source of enzymes with novel properties of high interest. Over the years, different enzymes of halotolerant and halophilic micro-organisms isolated from saline soils have been described and a number of new possibilities for industrial processes have emerged due to their overall inherent stability at high salt concentrations. These enzymes could be used in harsh industrial processes such as food processing, biosynthetic processes and washing (Ventosa et al., 2005).

Halopholic enzymes are active and stable at high salt concentrations, showing specific molecular properties that allow them to cope with osmotic stress. Mevarech et al. (2000) showed that these enzymes present an excess of acidic residues over basic residues and a low content of hydrophobic residues at their surface. Compatible

solute are low-molecular weight organic compounds such as polyols, amino acids, sugars and betaines that the halophilic and halotolerant bacteria accumulate intracellularly to achieve osmotic balance. On the other hand, halophilic bacteria tolerant to heavy metals could be used as bioassay indicator organisms in saline-polluted environments. Several halotolerant and halophilic bacteria isolated from hypersaline soils tolerate high concentrations of different metals such as Co, Ni, Cd, or Cr (Nieto et al., 1989; Rios et al., 1998).

In the biological treatment, the micro-organisms conventionally used show only poor degradative efficacy due to the highly saline conditions. The potential of halophilic organisms in effluent treatment offers the promise of innovative research. Other than that, halophilic is also used to recover saline soil by directly supporting the growth of vegetation, thus indirectly increasing crop yields in saline soil.

## DIVERSITY OF HALOPHILIC SOIL MICROORGANISMS

### Halophilic soil bacteria

The soil is an important habitat for bacteria. Soil bacteria can be found as single cells or as microcolonies, embedded in a matrix of polysaccharides. Bacteria inhabiting soil play a role in conservation and restoration biology of higher organisms. The domain bacteria contain many types of halophilic and halotolerant microorganisms, spread over a large number of phylogenetic groups (Ventosa et al., 1998). The different branches of the Proteobacteria contain halophilic representatives often having close relatives that are non-halophilic. Similarly, halophiles are also found among the cyanobacteria (Oren, 2002), the Flavobacterium - Cytophaga branch, the Spirochetes and the Actinomycetes. Within the lineages of Gram positive bacteria (*Firmicutes*), halophiles are found both within the aerobic branches (*Bacillus* and related organisms) as also within the anaerobic branches.

In general, it may be stated that most halophiles within the domain bacteria are moderate rather than extreme halophiles (Table 1). However, there are a few types that resemble the Archaeal halophiles of the family *Halobacteriaceae* in their salt requirements and tolerance.

Rodriguez-Valera (1988) stated that there was an abundance of halophilic bacteria in saline soil and that the dominant types encountered in saline soil belong to genera of *Alcaligenes*, *Bacillus*, *Micrococcus* and *Pseudomonas*. Garabito et al. (1998) isolated and studied 71 halotolerant Gram-positive endospore forming rods from saline soils and sediments of salterns located in different areas of Spain. These isolates were tentatively assigned to the genus *Bacillus*, and the majority of them were classified as extremely halotolerant

**Table 1.** Moderately halophilic bacteria.

S/N	Species	Gram's nature	Isolation Source	Reference
1	<i>Bacillus krulwichiae</i>	P	Soil from Tsukuba, Ibaraki, Japan	Yumoto et al. (2003)
2	<i>Bacillus haloalkaliphilus</i>	P	Showa, Saitama	Akinobu Echigo et al. (2005)
3	<i>Bacillus oshimensis</i>	P	Soil from Oshymanbe, Oshima, Hokkaido, Japan	Yumoto et al. (2003)
4	<i>Bacillus patagoniensis</i>	P	Rhizosphere of the perennial shrub <i>Atriplex lampa</i> in north-eastern Patagonia, Argentina	Olivera et al. (2005)
5	<i>Gracilibacillus halotolerans</i>	P	Shiki, Saitama	Akinobu Echigo et al. (2005)
6	<i>Halobacillus halophilus</i>	P	Salt marsh and saline soils	Spring et al. (1996)
7	<i>Halobacillus karajensis</i>	P	Saline soil of the Karaj region, Iran	Amoozegar et al. (2003)
8	<i>Halomonas anticariensis</i>	N	Soil from Fuente de Piedra. Málaga, Spain	Martínez-Cánovas et al. (2004)
9	<i>Halomonas boliviensis</i>	N	Soil around the lake Laguna Colorada, Bolivia	Quillaguaman et al. (2004)
10	<i>Halomonas maura</i>	N	Soil from a solar saltern at Asilah, Morocco	Bouchotroch et al. (2001)
11	<i>Halomonas organivorans</i>	N	Saline soil from Isla Cristina, Huelva, Spain	Garcia et al. (2004)

microorganisms, being able to grow in most cases in up to 20 or 25% salts.

Several alkaliphilic *Bacillus* species have been isolated from soil samples and it showed halophilic characteristics. *Bacillus krulwichiae*, a facultatively anaerobic (Yumoto et al., 2003), isolated in Tsukuba, Japan, is a straight rod with peritrichous flagella that produces ellipsoidal spores. These have ability to utilize benzoate or *m*-hydroxybenzoate as the sole carbon source. *Bacillus patagoniensis* (Olivera et al., 2005) was isolated from the rhizosphere of the perennial shrub *Atriplex lampa* in north-eastern Patagonia. Another is *Bacillus oshimensis* (Yumoto et al., 2005). It is a halophilic nonmotile, facultatively alkaliphilic species. Another example is the genus *Virgibacillus*. This genus comprises eight species, two of which are moderately halophilic and have been isolated from soil samples: *Virgibacillus salixigens* (Heyrman et al., 2003) and the recently described *Virgibacillus koreensis* (Lee et al., 2006).

Several other aerobic or facultatively anaerobic, moderately halophilic, endospore-forming, Gram-positive bacteria have been classified within genera related to *Bacillus*. Genera that include halophilic species isolated from soil samples are *Halobacillus*, *Filobacillus*, *Tenuibacillus*, *Lentibacillus* and *Thalassobacillus*. Species from *Filobacillus*, *Thalassobacillus* and *Tenuibacillus* genera are borderline halophile.

The genus *Halobacillus* is clearly differentiated from other related genera on the basis of its cell-wall peptidoglycan type. Within these genera, the halophilic species isolated from soils are: *Halobacillus halophilus* (Spring et al., 1996), *Halobacillus karajensis* (Amoozegar et al., 2003). With respect to the genus *Lentibacillus*, two halophilic soil species are identified. A *Lentibacillus salicampi* isolated from a salt field in Korea (Yoon et al., 2002), and A *Lentibacillus salarius* from a saline sediment

in China (Jeon et al., 2005).

The family *Nocardiopsaceae* contains three genera, namely *Nocardiopsis* (Meyer, 1976), *Thermobifida* (Zhang et al., 1998) and *Streptomonospora* (Cui et al., 2001). Some examples of moderately halophilic species of the genus *Nocardiopsis* isolated from soil samples are: *Nocardiopsis gilva*, *Nocardiopsis rosea*, *Nocardiopsis rhodophaea*, *Nocardiopsis chromatogenes*, and *Nocardiopsis baichengensis* (Li et al., 2006). These all were isolated from saline sediment from Xinjiang Province, China.

From salt pans of Kovalam in Kanyakumari district of Kerala, India, Gram negative moderately halophilic bacteria like *Natranobacterium sp-1*. was identified in the study of the diversity over a period of time (Saju et al., 2011).

Many Gram-negative, moderately halophilic, or halotolerant species are currently included in the family *Halomonadaceae*. This family includes three genera with halophilic species: *Halomonas*, *Chromohalobacter* and

*Cobetia*. Among the genera that comprise this family, *Halomonas* covers more than 40 species having heterogeneous features. Some species were isolated from soil samples: *Halomonas organivorans*, these species originating from saline soil samples in Spain, *Halomonas boliviensis* (Quillaguaman et al., 2004) were described as alkaliphilic and alkalitolerant, moderately halophilic bacteria, respectively, in as much as these bacteria are able to grow in media with pH values of about 8 to 9.

The genus *Marinobacter*, with the species *Marinobacter hydrocarbonoclasticus*, was created in 1992 to accommodate Gram-negative, moderately halophilic, aerobic Gammaproteobacteria that utilize a variety of hydrocarbons as the sole source of carbon and energy (Gauthier et al., 1992). It also accommodates moderately

halophilic *Marinococcus halophilus* and *Marinococcus albus* (Hao et al., 1984). Li et al. (2005) described a third species, *Marinococcus halotolerans* that is extremely halophilic. They are motile cocci that grow over a wide range of salt concentrations and up to 20% NaCl. The genus comprises 13 species, some of which are moderately halophilic bacteria isolated from soil samples: *Marinobacter lipolyticus*, that shows lipolytic activity with potential industrial applications (Martin et al., 2003), *Marinobacter excellens* (Gorshkova et al., 2003), *Marinobacter sediminum* (Romanenko et al., 2005), and the recently described *Marinobacter koreensis* (Kim et al., 2006).

### Fungal diversity in saline environment

The importance of halophilic fungi, long neglected as members of hypersaline ecosystems, became recognized only in the past decade. Gunde-Cimerman (2009) (Ljubljana, Slovenia) gave an overview of the biology of the most widespread and most halophilic or halotolerant fungi and yeasts. These include the black yeasts *Hortaea werneckii* which grows up to 5 M NaCl, the true halophile *Wallemia ichthyophaga* that requires at least 1.5 M NaCl and grows up to saturation, and *Aureobasidium pullulans* that grows up to 3 M NaCl. According to Gunde-Cimerman et al. (2009), the recent definition of halophilic fungi are those species which are isolated regularly with high frequency on selective saline media from environments at salinities above 10% and are able to grow *in vitro* on media with at least 17% NaCl.

All of these are commonly found in hypersaline lakes and a great variety of other, often unexpected, environments: domestic dishwashers, polar ice, and possibly even on spider webs in desert caves (Gunde-Cimerman et al., 2009). In fungi, a low osmotic potential decreases spore germination and the growth of hyphae and changes the morphology (Juniper and Abbott, 2006) and gene expression (Liang et al., 2007), resulting in the formation of spores with thick walls. Fungi have been reported to be more sensitive to osmotic stress than bacteria. There is a significant reduction in the total fungal count in soils salinized with different concentrations of sodium chloride.

The halophilic and halotolerant fungi use polyols such as glycerol, erythritol, arabitol and mannitol as osmotic solutes and retain low salt concentrations in their cytoplasm. Molecular studies on osmotic adaptation of *Hortaea werneckii* and *Wallemia ichthyophaga* were presented by Plemenitas et al. (2014) (Ljubljana, Slovenia). Identification and structural features of Na<sup>+</sup>-sensitive 3'-phosphoadenosine-5'-phosphatase HwHal2, one of the putative determinants of halotolerance in *H. werneckii* and a promising transgene to improve halotolerance in crops, was presented (Vaupotic et al., 2007). An in-depth

understanding has been obtained on the high osmolarity glycerol (HOG) pathway, and this understanding may be applied in the future on the development of improved salt-resistant crops.

Yeasts and other fungi are chemoheterotrophic cell-walled eukaryotes, some of which are well adapted to tolerate hypersaline environments. They grow best under aerobic conditions on carbohydrates at moderate temperatures and acidic to neutral pH. *Debaromyces hansenii* is a halotolerant yeast, isolated from sea water, that can grow aerobically in salinities of up to 4.5 mol/L NaCl. It produces glycerol as a compatible solute during the logarithmic phase and arabitol in the stationary phase. A saprophytic hyphomycete, *Cladosporium glycolicum*, was found growing on submerged wood panels at a salinity exceeding 4.5 mol/L NaCl in the Great Salt Lake. Halophilic fungi, e.g. *Polypaecilum pisce* and *Basipetospora halophila*, have also been isolated from salted fish (DasSarma and Arora, 2001).

### Vesicular arbuscular mycorrhiza

VA-mycorrhizal fungi occur naturally in saline environment (Khan and Belik, 1994). Several researchers investigated the relationship between soil salinity and occurrence of mycorrhizae on halophytes. They reported that the number of VAM spores or infectivity of VAM fungi changed with change in salt concentration (Juniper and Abbott, 1993). The stresses due to saline soils effect the growth of plants, fungus or both.

VA-mycorrhizal fungi most commonly observed in saline soils are *Glomus* spp. (Juniper and Abbott, 1993) this suggest that this may be adapted to grow in saline conditions, but ecological specificity has not been demonstrated. There is evidence that VAM species distribution is markedly changed with increased salinity (Stahl and Williams, 1986).

Aliasgharzadeh et al. (2001) observed that the most predominant species of arbuscular mycorrhizal fungi (AMF) in the severely saline soils of the Tabriz plains were *Glomus intraradices*, *G. versiform* and *G. etunicatum*. The authors also found that the number of AMF spores did not significantly decrease with soil salinity and reported a relatively high spore number (mean of 100 per 10 g soil). The higher fungal spore density in saline soils may be due to the fact that sporulation is stimulated under salt stress (Tressner and Hayes, 1971) which means that AMF may produce spores at low root-colonization levels in severe saline conditions (Aliasgharzadeh et al., 2001). Landwehr et al. (2002) reported abundant occurrence of AMF spores in extremely alkaline soils of pH values up to 11, independently of the soil type and irrespective of NaCl, Na<sub>2</sub>CO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub> or CaSO<sub>4</sub> salt types, though the degree of colonization varied from one individual to the next.

In most of the earlier studies, identification of the AMF spores was based mainly on the morphological criteria. Complementary to morphology based identification methods, use of molecular techniques such as polymerase chain reaction and restriction fragment length polymorphism for identification of AMF has been on the rise.

There are few studies indicating that mycorrhizal fungi can increase growth of plants growing in saline habitats (Ojala et al., 1983; Pond et al., 1984). VA-mycorrhizal fungi may have the ability to protect plants from salt stress, but the mechanism is not fully understood. The few data available at present suggest that fungi do have a potential to enhance plant growth by increasing the uptake of the nutrients. Recently, Porras-Soriano et al. (2009) tested the efficacy of three species of AMF – *Glomus mosseae*, *Glomus intraradices* and *Glomus claroideum* – to alleviate salt stress in olive trees under nursery conditions. The authors observed that *G. mosseae* was the most efficient fungus in terms of olive tree performance and particularly in the protection offered against the detrimental effects of salinity. These findings suggest that the capability of AMF in protecting plants from the detrimental effects of salt stress may depend on the behaviour of each species.

### Mechanisms for halotolerance

Halotolerance is the adaptation of living organisms to conditions of high salinity. High osmolarity in hypersaline conditions can be deleterious to cells since water is lost to the external medium until osmotic equilibrium is achieved. Many microorganisms respond to increase in osmolarity by accumulating osmotica in their cytosol, which protects them from cytoplasmic dehydration (Yancey et al., 1982). As biological membranes are permeable to water, all microorganisms have to keep their cytoplasm at least isoosmotic with their environment to prevent loss of cellular water; when a turgor pressure is to be maintained, the cytoplasm should even be slightly hyperosmotic. Adaptation to conditions of high salinity has an evolutionary significance. The concentration of brines during prebiotic evolution suggests haloadaptation at earliest evolutionary times (Dundas, 1998). Osmophily is related to the osmotic aspects of life at high salt concentrations, especially turgor pressure, cellular dehydration and desiccation. Halophily refers to the ionic requirements for life at high salt concentrations.

Halophilic microorganisms usually adopt either of the two strategies of survival in saline environments: 'compatible solute' strategy and 'salt-in' strategy (Ventosa et al., 1998). When an isoosmotic balance with the medium is achieved, cell volume is maintained. Compatible solute strategy is employed by the majority of moderately halophilic and halotolerant bacteria, some yeasts, algae and fungi. In this strategy, cells maintain low concentrations of salt in their cytoplasm by balancing

osmotic potential through the synthesis or uptake of organic compatible solutes and exclusion of salts from cytoplasm as much as possible. The compatible solutes or osmolytes, small organic molecules that are soluble in water to molar concentrations, which accumulate in halophiles are available in great spectrum and used in all three domains of life. These are assigned in two classes of chemicals: (1) the amino acids and their derivatives, such as glycine betaine, glutamine, glutamate, proline, ectoine or N-acetyl- $\beta$ -lysine and (2) polyols e.g. glycine betaine, ectoine, sucrose, trehalose and glycerol, which do not disrupt metabolic processes and have no net charge at physiological pH. The accumulation can be accomplished either by uptake from the medium or by *de novo* synthesis (Shivanand and Mugeraya, 2011).

The salt-in strategy is employed by true halophiles, including halophilic archaea and extremely halophilic bacteria. These microorganisms are adapted to high salt concentrations and cannot survive when the salinity of the medium is lowered. They generally do not synthesize organic solutes to maintain the osmotic equilibrium. In this adaptation, the intracellular  $K^+$  concentration is generally higher than that of outside, the intracellular  $Na^+$  concentration is generally lower than that in the medium, the intracellular  $K^+$  concentration increases with increasing external NaCl concentration in a non-linear pattern. All halophilic microorganisms contain potent transport mechanisms, generally based on  $Na^+ / H^+$  antiporters (Oren, 1999).

*Halobacillus* is the first chloride-dependent bacterium reported, and several cellular functions depend on  $Cl^-$  for maximal activities, the most important being the activation of solute accumulation. *Halobacillus* switches its osmolyte strategy with the salinity in its environment by the production of different compatible solutes. Glutamate and glutamine dominate at intermediate salinities, and proline and ectoine dominate at high salinities. Chloride stimulates expression of the glutamine synthetase and activates the enzyme. The product glutamate then turns on the biosynthesis of proline by inducing the expression of the proline biosynthetic genes (Saum et al., 2012). *Halobacillus dabanensis* is used by in Beijing, China as a model organism to study the genes involved in halotolerance, including genes encoding  $Na^+ / H^+$  antiporters, enzymes involved in osmotic solute metabolism and stress proteins (Yang et al., 2006).

### Applications of halophilic bacteria

Halophilic bacteria provide a high potential for biotechnological applications for at least two reasons: (1) their activities in natural environments with regard to their participation in biogeochemical processes of C, N, S, and P, the formation and dissolution of carbonates, the immobilization of phosphate, and the production of growth factors and nutrients (Rodriguez-Valera, 1993); and (2) their

nutritional requirements are simple. The majority can use a large range of compounds as their sole carbon and energy source. Most of them can grow at high salt concentrations, minimizing the risk of contamination. Moreover, several genetic tools developed for the nonhalophilic bacteria can be applied to the halophiles, and hence their genetic manipulation seems feasible (Ventosa et al., 1998).

Halophilic bacteria have the ability to produce compatible solutes, which are useful for the biotechnological production of the osmolytes. Some compatible solutes, especially glycine, betaines, and ectoines, may be used as stress protectants against high salinity, thermal denaturation, desiccation and freezing as well as stabilizers of enzymes, nucleic acids, membranes and whole cells. The industrial applications of these compounds in enzyme technology are most promising. The other compatible solutes such as trehalose, glycerol, proline, ectoines, sugars and hydroxyectoine from halophilic bacteria showed the highest efficiency of protection of lactate dehydrogenase against freeze-thaw treatment and heat stress.

Also, halophilic bacteria produce a number of extra- and intra-cellular enzymes and antimicrobial compounds that are currently of commercial interest (Kamekura and Seno, 1990). Halophilic bacteria can produce enzymes that have optimal activity at high salinity, which is advantageous for harsh industrial processes.

The application of halophilic bacteria in environmental biotechnology is possible for (1) recovery of saline soil, (2) decontamination of saline or alkaline industrial wastewater, and (3) the degradation of toxic compounds in hypersaline environments.

The use of halophilic bacteria in the recovery of saline soils is based on the hypothesis that microbial activities in saline soil may favor the growth of plants under salt stress. The second hypothesis is based on the utilization of these bacteria as bio-indicators in saline wells. Indicator microorganisms can be selected by their abilities to grow at different salt concentrations.

These organisms could be indicated that well water could be used for producing low saline contamination of plants or soils which could be alleviated desertification of soil (Ramos- Cormenzana, 1993). Last hypothesis is the application of halophilic bacterium genes using a genetic manipulation technique to assist wild type plants to adapt to grow in saline soil by giving them the genes for crucial enzymes that are taken from halophiles.

## CONCLUSIONS

Several studies have increased our current knowledge on different aspects of halophilic microorganisms from salted soils, such as their physiology, ecology, taxonomy or phylogenetic relationships with other microorganisms. Few hypersaline environments have been carefully surveyed using molecular methods. The recent finding on bacterial and archaeal metabolic activities suggests that

these environments may harbour diverse consortia of microbes that are not easily cultured. The extremes of hypersaline environments, may yield especially interesting species. There are a number of challenges involved in describing microbial dynamics in saline soil habitats that remain unsolved. There are so many literatures that provide the basic information for the study of halophilic bacteria in saline soil including the methodology for studying halophilic bacterial density and diversity.

Aforementioned studies show that flood tolerant plants and VA mycorrhizal endobiont fungi exist together and these fungi may play role in alleviating plant stress brought caused by flooding under aquatic environments. Some recent studies suggest that VA mycorrhizal endobiont would benefit both wetland and upland rice. But there are many biological principles such as infection strategy, nutrient uptake in hypersaline conditions that need to be explained. Besides, there are several fields in which their industrial applications are more promising, and, as in the case of other extremophilic microorganisms, halophiles have an important biotechnological potential as a source of compatible solutes, enzymes and other compounds of industrial interest. Moreover, moderately halophilic bacteria constitute an excellent model for the molecular study of the osmoregulatory mechanisms that permit them to grow over a wide range of salt concentrations. This aspect has very exciting potentialities. The genes involved in synthesis and accumulation of compatible solutes and their regulation have become the focus of recent investigations, for example, their possible application in agriculture to construct salt-resistant plants carrying prokaryotic genes encoding enzymes for the synthesis of osmoprotective compounds.

It is thus possible that in the future the biotechnological application of halophiles, or of genes derived from them, will extend to many more members of this extremely diverse group of microbes. Possible areas of exploitation may stretch from production of valuable compounds and remediation of contaminated waters and soils to future solutions of the world's problems.

Halophiles are likely to provide significant opportunities for biotechnology. As a result of natural and man-made global changes, hypersaline environments are on the increase. Moreover, hypersaline environments may easily be created by the concentration of sea water in arid environments. These facts, together with the occurrence of novel and stable biomolecules in halophiles, suggest that these organisms will prove even more valuable in the future.

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