**REVIEW ARTICLE**

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# Diversity, adaptation and activity of the bacterial flora in saline environments

**Abstract** Saline environments have a natural bacterial flora, which may play a significant role in the economy of these habitats. The natural saline environments (usually containing salinity equivalent to 4–30% NaCl) are aquatic (e.g. salt marshes) or terrestrial (e.g. saline lands). Saline environments include an increasing area of salt-affected cultivated soils throughout the world. These environments contain various ions which may interfere with uptake of water and which may be toxic to a large number of organisms. Saline environments harbour taxonomically diverse bacterial groups, which exhibit modified physiological and structural characteristics under the prevailing saline conditions. The majority of these bacteria can osmoregulate by synthesizing specific compatible organic osmolytes such as glutamine, proline and glycine betaine and a few of them accumulate inorganic solutes such as $Na^+$, $K^+$ and $Mg^{2+}$. The morphology of the bacteria is usually modified, cells are usually elongated, swollen and showing shrinkage, in addition to changes in the cell and cytoplasmic volume. The chemical composition of membranes may also occasionally be modified, and the synthesis pattern of proteins, lipids, fatty acids and polysaccharides may change with a moderate increase in salinity. However, ultrastructural alterations in cells of halophilic bacteria have not been reported, and profound changes in cellular properties of these bacteria only occur at concentrations above $2\,M$ NaCl. Evidence has accumulated that the bacteria are essential elements in the saline environment because of their activity such as degradation of plant remains, nitrogen fixation and production of active metabolites.

**Key words** Bacterial flora · Salt-affected soils · Salt marshes · Osmotic adjustment · Microbial activity · Nitrogen fixation

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## 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. The bacteria that live in saline environments may be assigned to two categories: archaebacteria and eubacteria. Archaebacteria are extremely halophilic microorganisms which grow optionally at salt saturation (up to 30% NaCl). It has been proposed, on the basis of many biochemical studies such as cell wall composition, ribosomal RNA sequence comparisons, and examination of the genetic organization and transcription of rRNA operons (Kjems et al. 1990; Dennis 1991; Denner et al. 1994), that the archaebacteria are a phylogenetically distinct group of microorganisms which are different from the eubacteria and the eucaryotes. Therefore, the archaebacterial group was suggested to form a separate kingdom, the Archaea (Woese et al. 1990). The archaebacterial flora will not be included in this review, although comparisons will be found elsewhere. The review discusses the characteristics of the bacterial flora inhabiting the saline environments, their diversity and adaptation and the microbial activity of these environments. The bacterial communities in saline environments may include extreme (halophilic) and facultative (halotolerant) bacteria. The latter may include the nonsaline bacteria, which have become adapted to these extreme environments. However, this review is not devoted to halophilic bacteria (organisms which are almost restricted to extreme environments), but aims mainly to present a comprehensive picture of the whole bacterial flora of various saline environments and their free-living or associative activity especially with halophytic plants.

## Saline environments and their vegetation

### Saline lands and salt-affected soils

Saline lands include salt desert, which cover huge areas of the world, where evaporation greatly exceeds precipitation

(Fitter and Hay 1987). There may be salination to such a degree as to eliminate most plants from these habitats. Saline lands represent about 15% of the arid and semiarid lands of the world, while salt-affected soils represent about 40% of the world's irrigated lands (Hoffman et al. 1980; Moore 1984; Shannon 1984; Serrano and Gaxiola 1994). Reliable estimates suggest that in the Mediterranean region alone, about 15 million hectares have become seriously affected due to man's activities (Aronson 1985). In Egypt, saline lands and sodic soils on regularly irrigated lands and the waste saline lands to the south of the Nile Delta have dramatically increased. Recent reports estimated salt-affected lands (mostly agricultural land) to be about 1.5 million hectares (Zahran et al. 1992). The main reasons for the salinity problem in the agricultural land of Egypt are: (a) the use of salt-contaminated irrigated water, (b) poor drainage management, and (c) the rise of underground water after the Nile flooding stopped because of the construction of the High Dam (Zahran et al. 1992). In India, over 6 million hectares of a total of 40 million hectares of irrigated farmland have been made useless by salinity and waterlogging, in addition to about 40 000 ha which are abandoned every year (Chapman 1975). In the United States (California) about 0.5 million hectares have been strongly affected by salinity (Kelley et al. 1979). It has been reported (Yensen et al. 1981) that the United States alone loses about 80 000–120 000 ha crop land every year due to salt build-up. This is due to the cumulative effects over decades or centuries of adding water with some dissolved salt to the soil of arid regions (Downton 1984).

Salinity usually exists in soil as the underground water rises by capillarity or the rain water accumulates in low places without drainage. High temperature increases the rate of evaporation of water, leaving crusts of salts above the soil surface or crystals of salt which may be found in the upper layers mixed with the soil mineral constituents. In these soils there is a high concentration of neutral soluble salts (ECe 5 ds cm$^{-1}$ or more) sufficient to seriously interfere with microbial activity and soil fertility (Sarig et al. 1993) and also the growth of most plants (Zahran 1989). The salinization of the soil is very dangerous for soil productivity, when there is no effective means to deal with the salinity problem. The Sumerian civilization in Mesopotamia vanished because they failed to respond to this threat (Jacobson and Adams 1958). Some engineering technologies, e.g. drainage of salinized water and supplying high-quality water from remote sources, have been suggested but they are extremely costly (Zahran 1991; Serrano and Gaxiola 1994). The following strategies have been suggested (Aronson 1985; Zahran 1991) to improve the productivity of saline lands and plant growth: (a) improvement of the rate of drainage so that salt build-up is slowed, (b) selection or breeding for greater salt tolerance of conventional crops showing some genetic variability for salt tolerance, and (c) the use of potentially useful wild halophytes. Halophytes which are of significant agricultural or horticultural value have been used for land reclamation in many countries of the world (Aronson 1985). Nitrogen

fixation, in association with halophytes (Wollenweber and Zechmeister-Boltenstern 1989) or symbiotically with salt-tolerant legumes (Zahran 1991), may increase N input in saline soils and therefore could be a significant solution to the aridity of saline lands.

Aquatic saline environments

Concentrated salt solutions (brines) are found in natural ecosystems such as salt lakes, marine ponds and salt marshes subject to evaporation because of high temperature (Trüper and Galinski 1986). The inland lakes of the world may include some of the most extreme natural environments, e.g. Dead Sea, Great Salt Lake, Soda Lake and salt marshes (Batanouny 1979; Zahran 1989; Ollivier et al. 1994). Salt marshes may be found in inland areas and coastal (marine) marshes occur in sheltered sites (frequently estuaries) where wave action is slight and deposition of silt allows higher plant to root. The estuaries and particularly shoreline rockpods exposed to intensive evaporation can also become extremely saline (Ollivier et al. 1994). Human activity also creates highly saline habitats such as solar salterns, which may have an NaCl concentration at saturation in some ponds (Trüper and Galinski 1986; Ollivier et al. 1994). Inland and marine salt marshes, as saline habitats, are widely distributed in the western and eastern deserts of Egypt (Batanouny 1979; Zahran 1989; Zahran and Willis 1992). The inland salt marshes which are far from the reach of maritime influences are present in the oases and depressions of the deserts (Zahran and Willis 1992). The marine (littoral or coastal) salt marshes usually occupy the lands adjacent to maritime influences (i.e. periodic tidal flooding, seawater spray and seawater seepage) along the coasts of the Mediterranean Sea, Red Sea and also the northern lakes (Zahran and Willis 1992).

Physicochemical characteristics of saline environments

The hypersaline ecosystems such as salt lakes and marshes show a great variability in total salt concentration, ionic composition and pH (Ollivier et al. 1994). Several lakes, e.g. Big Soda Lake, Mono Lake and Soap Lake, in the Great basin of the western United States, have salinities ranging from about 9 to 10% (wt/vol) and are highly alkaline with pH values of 9–10 (Oremland and King 1989). In contrast, the saline water bodies, e.g. the Great Salt Lake, the Dead Sea and the Orea basin in the Gulf of Mexico, which are hypersaline with total salt contents of over 20%, have pH values of around 7.0 (Oremland and King 1989). In these saline solutions, the $Na^+$ and $Cl^-$ ions are predominant (Oren 1993).

In the Great Salt Lake, the concentrations of $Na^+$ and $Cl^-$ are about 10.5% and 18.2%, respectively. In the Dead Sea $Cl^-$ ions are dominant, being about 22.5%, with equivalent concentrations of $Na^+$, $Mg^{2+}$ and $Ca^{2+}$ of about 4%, 4.4% and 1.7%, respectively. The absence of or the low concentra-

tions of divalent cations such as $Mg^{2+}$ and $Ca^{2+}$ from brackish water or brines is mainly attributed to the high pH of these hypersaline ecosystems (Ollivier et al. 1994). The sulphates, which are important electron acceptors and are involved in the mineralization of organic matter, are present at low concentrations in hypersaline waters (about 0.05% in the Dead Sea, 0.27% in Great Salt Lake and 0.21% in the Soap Lake) probably because of its precipitation (Oremland and King 1989; Ollivier et al. 1994).

The salinity of salt-affected soils of Egypt was found to be about 1–3% (w/v) and the organic matter content 1–4.5% (Zahran et al. 1992; Zahran et al., unpublished). In saline soils of Egypt, the cations were commonly $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$, while the anions were mainly chlorides, with few sulphates, nitrates, carbonates and bicarbonates (Zahran 1989). The pH of saline soils is usually less than 8.5 because the soluble salts present are mainly neutral and the amount of the exchangeable sodium is small (less than 15%). In addition, these soils contain variable amounts of ammonia and nitrate. The hypersaline soil in India (Chawla 1969) and Spain (Del Moral et al. 1987), on the other hand, is characterized by a low organic matter content. Therefore, it has been suggested (El Abyad et al. 1979; Zahran et al. 1992; Ragab 1993) that salinity is not the only factor which determines the characteristics of saline soils but also the organic matter content. The type and density of vegetation affect the organic matter content in saline soils, and higher organic matter content was found in the rhizosphere of halophytic plants, which are common in saline habitats (Batanouny 1979; Quesada et al. 1982; Zahran 1989). The existence of bacteria in saline soils might therefore be affected by the organic matter content. Saline soils contain variable amounts of combined N, and higher nitrate contents are found in salt-affected soils in Egypt (Zahran et al. 1992; Zahran et al. unpublished), probably because of the extensive application of N fertilizer. Nitrates may be depleted from saline lands as a consequence of higher rates of denitrification (Kaplan et al. 1979); in addition, the halophytic plants usually act as a scavenger to nitrates and ammonia accumulates in these habitats (Lee et al. 1989). Ammonia usually accumulates in saline lands as a product of the nitrogen fixation process (Yoch and Whiting 1986; Wollenweber and Zechmeister-Boltenstern 1989). Nitrogen-fixing bacteria were isolated from saline soils of Egypt (Zahran et al. 1995). The existence of bacterial communities in saline soils may also be affected by alkalinity. The pH of saline soils was about 8.5 in Spain (Quesada et al. 1982), 10.5 in India (Bhardwaj 1974) and 8.8 in Egypt (Ragab 1993; Zahran et al., unpublished). The wet soils of the marshes or lake shores have moderate salinity and alkalinity compared to the marsh or lake solution itself, which is usually extremely saline and alkaline (Imhoff et al. 1979).

Vegetation in saline environments

Halophytes, the vegetation of saline habitats, are a specialized plant group, characterized by the possession of great osmotic tolerance (Zahran 1989). A comprehensive survey of the halophytic vascular plants of the world revealed that more than 1250 species of halophytes (in at least 10 families) occur in saline lands and salt marshes (Aronson 1985). A halophyte is any plant that can complete its life cycle and reproduce itself under conditions of soil water-salinity of ECe 8–10 ds cm$^{-1}$ (about 0.08–0.1 $M$ NaCl). Halophytes are divided into two different types according to the type of modification or the sort of adaptation to saline environments: (1) the excretives (plants capable of excreting excess salts), including *Tamarix, Limonium* and *Limoniastrum*, and (2) the succulents (plants having succulent leaves), including *Suaeda, Salicornia, Zygophyllum, Arthrocnemum* and *Halocnemum.* The latter two species are dominant in Red Sea coastal salt marshes (Zahran 1989). The halophytes *Cyperus, Juncus* and *Typha* are also dominant in the swamp lands bordering the salt marshes in Egypt (Zahran and Willis 1992). Halophytes are usually found in mangrove swamps (shallow salt marshes) which occur sporadically in many arid and tropical coastal areas. Mangroves (mangal) communities occupy a zone of salt marshes of the Red Sea in Egypt, which is permanently covered with sea water (Zahran 1989). At least 50 tree and shrub species in 10 families and 14 genera are commonly accepted as mangroves, the major economic genera of worldwide importance being *Avicennia, Brugieria, Canocarpus, Heritiera, Laguncularia* and *Rhizophora* (Aronson 1985; Zahran 1989). Mangrove halophytes are an abundant source of timber, firewood, charcoal and tannins (Aronson 1985). The salinity in the mangrove swamps of the Red Sea is about 4–6.5% and the pH about 7–8 (Shoreit et al. 1994). The main bacterial flora in these habitats comprises members of four genera of purple non-sulphur bacteria *(Rhodopseudomonas, Rhodospirillum, Rhodobacter* and *Rhodocyclus).* In addition to the halophytes, there are some legumes which have been observed growing in salt-affected soils in the Middle and Far East and in other places. Other woody (tree) legumes are found in arid saline lands. If these leguminous plants (which are nitrogen-fixing) are grown in saline lands, they will improve the fertility of these soils (Zahran 1991). The salt tolerance of legume-*Rhizobium* symbioses and their nitrogen-fixing activity have been studied under saline conditions (Zahran 1991; El Sheikh and Wood 1995); a salt-tolerant strain of *Rhizobium* fixed more nitrogen with soybean plants than a salt-sensitive *Bradyrhizobium* in saline soil. Selection of salt-tolerant and nitrogen-fixing legumes (herb or woody) was a strategy suggested for cultivation of the saline lands in arid regions and the salt-affected soils, and for improvement of soil fertility (Zahran 1991).

## Diversity, taxonomy and characteristics of bacterial flora of saline environments

The total count of bacteria is usually negatively correlated with the total soluble salts of saline soils (Ragab 1993), but positively correlated with organic carbon contents (El-

Abyad et al. 1979; Zahran et al. 1992; Ragab 1993). However, these soils may have their own bacterial communities which have adapted to saline environments. Viable counts in plates cannot yield a reliable picture about the bacterial flora of saline soils, because the number of colonies yielded represents only a small percentage of the real number of colonies (Oren 1991). A significantly higher number of bacteria was found to colonize the hypersaline soils of Spain (Quesada et al. 1982; Del Moral et al. 1987) and Egypt (Zahran et al. 1992; Zahran et al., unpublished), when a medium with high salinity was used. The strict halophiles only develop in media with higher concentrations of $Na^+$, whereas the facultative halophiles usually develop in media containing low concentrations of $Na^+$ (Giambiagi and Lodeiro 1989). Therefore, Bhardwaj (1974) suggested using a medium with a high salt concentration and a high pH in order to obtain an accurate picture of the bacterial flora of saline soils.

The bacterial flora of saline environments is as diverse as its fresh-water counterpart (Galinski and Trüper 1994). Nevertheless, the saline soil bacteria do not define a group of phylogenetically related microbes but represent a group which has evolved in many different groups of organisms. The wide spectrum includes the following representatives, *Halomonas, Pseudomonas, Vibrio* and *Actinopolyspora* and a whole range of Gram-positive rods and cocci, e.g. *Bacillus, Micrococcus* and *Salinicoccus* (Trüper et al. 1991). The Gram-negative bacteria appear to be much more frequent in saline environments (Quesada et al. 1982; Del Moral et al. 1987). They were isolated from saline water and saline soils and have received much attention compared to other bacterial types of saline environments (Vreeland et al. 1980; Quesada et al. 1983, 1984, 1987; Oren et al. 1984; Rodriguez-Valera et al. 1985; Ventosa et al. 1985; Rengpipat et al. 1988 a, b; Zahran et al. 1992). Members of the Gram-negative genera, *Vibrio, Pseudomonas, Acinetobacter* and *Alteromonas*, have been isolated from saline habitats (Rosenberg 1983; Rodriguez-Valera et al. 1985; Del Moral et al. 1988; Zahran et al., unpublished). The root-nodule bacteria are another group of Gram-negative bacteria which have been reported to colonize the saline soil of Greece (Douka et al. 1978) and Egypt (Zahran et al. 1992).

The Gram-positive bacteria are also well represented in saline habitats, and members of the genera *Bacillus* and *Micrococcus* are dominant among other Gram-positive bacteria in saline soils. For example, members of the spore-forming *Bacillus* were isolated from the hypersaline habitats of Spain (Quesada et al. 1982) and Egypt (Weisser and Trüper 1985; Zahran et al. 1992, 1995). These spore-forming bacilli are mostly moderate or extreme halo-alkaliphiles. Gram-positive cocci have been isolated from various saline ecosystems (Quesada et al. 1982; Claus et al. 1983; Del Moral et al. 1987; Zahran et al. 1992). These bacteria were mainly included in five species, they are: *Marinococcus halophiles, M. albus, M. hispanicus, Micrococcus halophilus* and *Sporosarcina halophila* (Marquez et al. 1990).

The actinomycetes represent only a small fraction of the bacterial flora of saline soils (Quesada et al. 1982; Zahran et al. 1992; Zahran et al., unpublished); they may be less tolerant to salt stress than other bacterial types (Yokoyama et al. 1992). However, two extremely halophilic actinomycetes *(Actinopolyspora halophila* and *A. mortivallis)* were isolated from saline habitats (Johnson et al. 1986; Kates et al. 1987; Gochnauer et al. 1989; Yoshida et al. 1991). A new actinomycete was isolated from the saline soil of Iraq (Al-Tai and Ruan 1994). This organism grew best in the presence of 20% NaCl; the name *Nocardiopsis halophila* was proposed for the bacterium after physiological, morphological and biochemical studies. Another new halophilic actinomycete was isolated from the same saline soil of Iraq (Ruan et al. 1994) and the name *Actinopolyspora iraqiensis* was proposed for this isolate. These recent reports indicate that many other new actinomycetes may colonize saline soils; nevertheless, the isolation media must be carefully chosen and the cultural conditions should be optimized. Although most studies of saline environments have been limited to aerobic organisms, these ecosystems are also inhabited by a wide range of anaerobic microorganisms. The characteristics of a number of anaerobic bacteria such as the spore-forming bacterium *Clostridium halophilum* isolated from saline environments (Fendrich et al. 1990) have been studied, although to a much lesser extent than their aerobic counterparts (Lowe et al. 1993).

The interest in searching for new strains of anaerobic bacteria in the saline environments has increased, in order to understand the mechanisms involved in osmotic regulation and enzymatic activity at high salt concentrations (Cayol et al. 1994). The following strictly anaerobic Gram-negative bacteria, inhabiting various saline environments, have been recently described in more detail (Cayol et al. 1994; Ollivier et al. 1994); they include *Haloanaerobium prevalens, Halobacteroides halobius, Halothermothrix orenii, Sporohalobacter lortetii* and *S. marismortui*, from sediments, and *Halobacteroides acetoethylicus, Haloanaerobacter chitinovorans, Halobacteroides lacunaris, Haloincola saccharolytica, Acetohalobium arabaticum* and *Halocella cellulolytica*, from brine waters and lakes. A new anaerobic Gram-negative bacterium *(Haloanaerobium salsugo)* has been isolated from a highly saline oil field (Bhupathiraju et al. 1994). The bacterium had a specific requirement for NaCl and grew at NaCl concentrations between 6% and 24%, with optimal growth at 9% NaCl. The anaerobic bacteria of saline environments are differentiated on the basis of salt tolerance range, catabolism habit, DNA base composition and spore formation (Lowe et al. 1993) and also by 16S rRNA oligonucleotide-cataloging analysis (Cayol et al. 1994).

## Mode of life and tolerance to saline environments

The determination of saline spectrum is very important for the characterization of the type of bacteria to be regarded as halotolerant or extreme, moderate and slight halophiles. According to the salt concentration required for optimum

growth, the bacteria were classified (Imhoff 1986) as non-halophilic (grow below 0.2 $M$ NaCl), slightly halophilic (grow at 0.2 to about 1.0–1.2 $M$ NaCl), moderately halo-philic (grow at about 1.0–1.2 to 2.0–2.5 $M$ NaCl) and ex-tremely halophilic bacteria (grow at 2.0–2.5 $M$ NaCl or more). Halophily is demonstrated by the requirement of a high salt concentration for optimum growth; however, ha-lotolerance, qualitatively and quantitatively, describes the ability to grow at a salt concentration higher than optimum (Imhoff et al. 1991; Ollivier et al. 1994). However, the de-gree of tolerance of bacteria depends on the composition of the growth medium (Larsen 1986; Rodriguez-Valera 1988; Ramos-Cormenzana 1991). The halophilic eubacter-ia are usually found in numerous saline habitats with dif-ferent salinities, e.g. hypersaline soils (Quesada et al. 1982, 1983), inland marshes (Del Moral et al. 1988), bot-tom sediments (Oren et al. 1984) or other different habi-tats (Vreeland et al. 1980; Quesada et al. 1985). They are present in non-saline habitats, in the same manner that non-halophilic bacteria are present in hypersaline environ-ments (Marquez et al. 1987; Ramos-Cormenzana 1991). Therefore, two questions have been of great interest: do halophilic organisms survive in environments where there is a low salt concentration and, secondly, do the bacteria come from non-halophilic organisms by an adaptation pro-cess or did bacteria evolve from halophiles by an adapta-tion mechanisms to non-saline conditions (Ramos-Cormen-zana 1991)? A salt-sensitive mutant of the moderately ha-lophilic bacterium *(Vibrio costicola)* was isolated (Kogut et al. 1992); the mutant was not able to grow at high (i.e. 2.5–3.0 $M$) concentrations of NaCl in contrast to the wild-type strain, which normally grows at 3.4 $M$ NaCl.

In general, the bacteria have evolved two different stra-tegies for growth in an environment of concentrated NaCl (Lowe et al. 1993). The internal salt concentration can be maintained at a level comparable to that of the environ-ment, or the organism can actively exclude NaCl and pro-duce an organic compatible osmoregulant such as betaine (Trüper and Galinski 1986). The eubacteria may have a low internal salt concentration (Kushner 1989); however, the archaebacteria usually have an internal salt concentra-tion which is approximately equal to the outside concen-tration. The aerobic halophilic archaebacterium *Halobacter-ium salinarium*, growing in 4 $M$ NaCl plus 0.032 $M$ KCl, contains about 1.4 $M$ Na$^+$, 4.6 $M$ K$^+$ and 3.6 $M$ Cl$^-$, whereas the moderate halophilic eubacteria *Vibrio costico-la*, growing in 1 $M$ NaCl and 0.006 $M$ KCl, contains about 0.6 $M$ Na$^+$ and K$^+$, but only about 0.1–0.2 $M$ Cl$^-$ in its cells (Kushner 1989). The moderate halophilic eubacteria concentrate K inside the cell but exclude Cl$^-$, so that their internal ion concentration may be only about one-tenth that of the external concentration. However, the anaerobic halophilic eubacteria, *Haloanaerobium prevalens, Halo-bacteroides halobius* and *H. acetoethylicus* (Oren 1986; Rengpipat et al. 1988 a, b), maintain high intracellular con-centrations of salt and accumulate Cl$^-$ inside the cell, which is compatible to the activity of a number of en-zymes in the cell, but osmoregulants are not produced. These bacteria demonstrate a marked difference from ha-loaerobic eubacteria, which exclude Cl$^-$ from the cell. The cellular processes in bacteria from saline environments presumably have adapted to functioning in such an envi-ronment (Lowe et al. 1993). Comparison of the effect of salt on the in vitro activity of a number of enzymes from the anaerobic bacterium *Halobacteroides acetoethylicus* has revealed some striking similarities with enzymes from archaebacteria. However, studies on the effect of salt on enzymes from haloaerobic eubacteria have shown that most of the enzymes were active at salt concentrations be-low those found inside the cell.

## Adaptation of bacteria to saline environments

### Osmoregulation

Bacteria existing in saline environments have to cope with a number of stresses such as the ionic stress (Galinski and Trüper 1994). Increases or decreases in the extracellular osmolarity where bacteria are growing commonly elicit corresponding changes in the intracellular concentrations of compatible solutes (Csonka 1989). The nature and com-position of the medium affects the intracellular ion content and the osmoregulating ability of these bacteria. The bac-teria of the genus *Brevibacterium* accumulate higher Na$^+$ (ca. 10 times) when grown in defined medium compared to the complex medium (Nagata et al. 1991). Cells tend to keep their volume fairly constant over a large range of ex-ternal salinity (Csonka and Hanson 1991). Therefore, the cell membrane of the bacteria living in saline environ-ments forms the primary barrier displaying adaptive changes in the face of altering salinity (Imhoff and Thie-mann 1991; Thiemann and Imhoff 1991). The adaptation to osmotic stress requires an osmotic equilibrium across the membrane and a cytoplasm of similar osmotic strength to the surrounding medium (Larsen 1986; Trüper and Ga-linski 1986).

The bacteria living in saline environments have adopted two strategies for osmoadaptation (Galinski and Trüper 1994), the KCl type and the compatible solute type. In the first mechanism, the bacteria maintain a cytoplasmic KCl concentration similar to that of the surrounding medium in order to attain an osmotic equilibrium. 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 organ-elles) at high salinity (Eisenberg and Wachtel 1987). The KCl type of osmoregulation is adopted by members from archaebacteria and also eubacteria. The latter include the fermenting and/or acetogenic anaerobes *Haloanaerobium* and *Acetohalobium* (Zhilina and Zavarzin 1990; Oren 1991) and the sulphate reducers *Desulfovibrio halophilus* and *Desulfohalobium retbaense* (Gaumette et al. 1991; Ollivier et al. 1994). The compatible solute strategy of os-moadaptation, on the other hand, is also adopted by eubac-teria and a few members of the archaebacteria (Nicolaus et

al. 1989). The compatible solutes were described as organic osmolytes which are responsible for osmotic balance and compatible with the cell metabolism, and usually accumulate in the cytoplasm at concentrations well above $1\,M$ (Galinski and Trüper 1994). The accumulation and complement of organic solutes was found to require genetic induction (Smith et al. 1994a) and to depend on the level of osmotic stress, growth phase of the culture, carbon source, and the presence of osmolytes in the growth medium (Smith et al. 1994b). It has been proposed (Csonka and Hanson 1991; Lippert and Galinski 1992) that these organic solutes have two mechanisms for their mode of action under saline conditions: first to increase the intracellular osmotic strength, and second to stabilize cellular macromolecules. The compounds which have this ability are sometimes called osmoprotectants.

Compatible solutes can be accumulated in bacteria by de novo synthesis (Csonka 1989; Csonka and Hanson 1991) or by transport from the culture medium (Wohlfarth et al. 1990; Severin et al. 1992) to conserve energy (Galinski and Trüper 1994). When these solutes are added to the bacterial cultures, they may elicit a dramatic stimulation in growth rate of the cells in media of high osmolarity. Solutes which would alleviate osmotic inhibition should be accumulated to higher internal concentration. On the other hand, salt-sensitive bacteria (e.g. entric bacteria) have effective transport mechanisms for some osmolytes (e.g. betaine and ectoine); therefore, they can acquire a certain degree of halotolerance by uptaking compatible solutes which are sometimes present in marine sediments, or released from other bacteria into the environment and when these solutes are supplied into the medium (Koo et al. 1991; Cayley et al. 1992; Jebbar et al. 1992).

By using new analytical methods and modern techniques (e.g. high-performance liquid chromatography and nuclear magnetic resonance), the following classes of organic solutes were detected: glycine betaine, amino acids (e.g. proline and glutamine), N-acetylated amino acids, N-derivatized carboxamides of glutamine, sugars and sugar polyol derivatives (Trüper and Galinski 1986; Smith and Smith 1989; Wohlfarth et al. 1990; Severin et al. 1992; Galinski and Trüper 1994; Smith et al. 1994a,b), in addition to the newly discovered ectoines (Talibart et al. 1994). Several observations suggest that glycine betaine ($N$,$N$,$N$-trimethyl glycine) is preferred over all other compatible solutes for a large number of bacteria under conditions of hyperosmolarity (Imhoff and Rodriguez-Valera 1984). However, only few phototrophic ($CO_2$-fixing) species (Trüper and Galinski 1990) and cyanobacteria (Reed and Stewart 1985; Imhoff 1986) were able to synthesize glycine betaine. Bacteria such as some members of the Enterobacteriaceae (Le Rudulier and Bouillard 1983; Csonka 1989) and *Rhizobium* spp. (Le Rudulier and Bernard 1986; Fougere and Le Rudulier 1990) accumulate glycine betaine by transport from the external medium. Glycine betaine was found to stimulate recovery of a lost volume of *Brevibacterium lactofermentum* and *Corynebacterium glutamicum* when subjected to hyperosmotic stress (Skjerdal et al. 1995). The cytoplasmic level of glutamate usually increases in most procaryotes after exposure to media of high osmolarity (Hua et al. 1982; Yap and Lim 1983; Botsford 1984; Botsford and Lewis 1990; Fujihara and Yoneyama 1993); osmotic stress can elicit over tenfold increases in the levels of glutamate in Gram-negative bacteria. The relative increases in glutamate in Gram-positive bacteria are much less than that of Gram-negative bacteria (Killham and Firestone 1984).

Proline is an important osmoprotectant for several bacteria. Some of the Gram-positive bacteria are able to increase the proline pool size by synthesis upon exposure to osmotic stress in the absence of exogenous proline or by transport from the external medium (Koujima et al. 1978). Gram-negative bacteria (e.g. *E. coli, Klebsiella pneumoniae* and *Salmonella typhimurium*) are entirely dependent on the presence of the exogenous proline for osmotic adjustment; they usually accumulate high concentrations of proline under osmotic stress (Le Rudulier and Bouillard 1983; Csonka 1989). The occurrence of sugars such as sucrose and trehalose appear to be common in some microorganisms when they grow under osmotic stress. These sugars rarely exceed a cytoplasmic concentration of about $0.5\,M$ (Galinski and Trüper 1994). However, the available sugars affect the relative proportions of solutes, e.g. proline, in some bacteria, e.g. *Deleya* and *Flavobacterium* spp. (Wohlfarth et al. 1990) and *Rhizobium* (Ghittoni and Bueno 1995, 1996), at high salt concentration. Sugars are less compatible solutes and are usually used for osmotic adaptation in limited salt-tolerant organisms, since it has been found that addition of sugars (e.g. sucrose) to the normally growing bacteria resulted in physiological and structural alterations (Knowles and Smith 1971; Parente and Silva 1984).

## Modifications in cell morphology and structure

The bacteria which usually grow in non-saline conditions may exhibit a great modification in cell morphology when subject to high salt stress. The swelling, elongation and shrinkage (reduction in cell volume) are characteristic features of sensitive bacteria under salt stress. However, it has been recently found (Skjerdal et al. 1995) that the cell and the cytoplasmic volume of *Brevibacterium lactofermentum* and *Corynebacterium glutamicum* (a salt-tolerant bacteria) spontaneously decrease upon hyperosmotic shock. The modification in cell morphology under salt stress is a familiar response of some Gram-negative bacteria such as *Azotobacter vinelandii* (Knowles and Smith 1971), *E. coli* (Baldwin et al. 1988), *Pseudomonas fluorescens* (Parente and Silva 1984) and *Rhizobium* (Zahran et al. 1993). The root-nodule bacteria of the genus *Rhizobium*, isolated from salt-affected soils, have shown some salt-induced alterations (Zahran 1991b, 1992a,b). The cell shape, the synthesis pattern of protein and lipopolysaccharide as well as the genomic structure of these bacteria were modified. These alterations may affect the symbiosis between rhizobia and their legume hosts. The morphology of some Gram-negative bacteria of saline soil has been re-

ported to be modified (Zahran et al., unpublished); cells were elongated several times when subjected to 10–20% NaCl. The Gram-positive bacteria (e.g. *Bacillus* and *Staphylococcus* modified their cell structure under salt stress conditions. Cells of *Bacillus* were elongated and thickened, and the formation of more chains (streptobacilli) was stimulated at 10% NaCl, and the cell volume of *Staphylocuccus* was reduced at 10–20% NaCl (Zahran et al., unpublished). Heat stress brings about similar effects; cells of *Bacillus* were elongated about 5–6 times more than the normal size and became filamentous at 45 °C (Kudo and Horikoshi 1979). The formation of endospores in cells of *Bacillus*, which are usually controlled by nutrient availability and cultural conditions (Stock et al. 1989), was also affected by alkalinity and salt stress (Kudo and Horikoshi 1979; Weisser and Trüper 1985; Zahran et al. 1992). The bacilli isolated from saline soils, however, formed endospores at 5–20% NaCl (Zahran et al., unpublished); these endospores were formed at salt concentrations which are optimal for the growth of these bacteria (Horikoshi and Akiba 1982).

Salt-tolerant bacteria usually exhibit structural modifications to cope with salt stress. One important aspect of structural adaptations is the change in composition of the cell envelope and membranes. The stretched state of the wall and the internal osmotic pressure of bacteria are usually affected by the biophysical properties of the stress-bearing peptidoglycan (Koch 1984). Changes in composition of bacterial membranes which might be caused by environmental factors are thought to act as an adaptive response to maintain membrane stability and function (Imhoff and Thiemann 1991). In fact, structural adaptations of membranes mainly involve alterations in the composition and synthesis of proteins, lipids and fatty acids (Kates 1986; Russell 1989; Thiemann and Imhoff 1991). Some bacteria may occasionally show slight cell modifications, but more profound changes in cellular properties of bacteria only occur at concentrations above $2 M$ NaCl as an adaptation to salt stress (Kogut 1991). Electron micrographs of thin sections, however, have not revealed any ultrastructural differences in membranes of the anaerobic bacteria *(Halobacteroides halobius* and *H. acetoethylicus)* that could account for the ability of these bacteria to inhabit ecological niches high in salt (Lowe et al. 1993). Those two species of *Halobacteroides* had a typical cell envelope with characteristic layering of Gram-negative bacteria, but the latter species contained unusual crystal-like forms with unknown function (Rengpipat et al. 1988 a, b; Lowe et al. 1993).

The properties of bacterial membranes, to a great extent, depend on their lipid composition. The polar lipids approximate about 75–93% of the total lipid carbon in cell of the halophilic bacterium *Ectothiorhodospira* (Thiemann and Imhoff 1991); the negatively charged phospholipids of *Ectothiorhodospira* usually increase upon increasing medium salinity. The increase in the proportion of the negatively charged lipids in cell membranes of bacteria is a common feature of the adaptive responses to elevated external salinity (Russell and Adams 1991; Thiemann and

Imhoff 1991). *Halomonas elongata* is able to live in a wide variety of salt concentrations (0.05–3.4 $M$ ) because it alters the cell physiology in ways which increase the structural integrity of walls and increase amounts of negatively charged lipids (Vreeland et al. 1984, 1991). The halophilic bacteria, e.g. *Haloanaerobium prevalense*, however, show different responses and the lipid content of the cell is not changed under salt stress (Zeikus et al. 1983). The fatty acid composition of the individual phospholipids, normally regulated by culture-medium salinity, is important in maintaining a stable bilayer structure within the membrane of bacteria grown under salt stress (Imhoff and Thiemann 1991; Sutton et al. 1991). The stability of the bilayer is of crucial importance for cell viability. There are other molecules with low molecular weight, which have been identified, e.g. in *E. coli* (Kennedy 1982), as membrane-derived oligosaccharides. They accumulate in the periplasmic space of Gram-negative bacteria, and play an effective role in cell modification under salt stress (Imhoff 1986).

## Activities of bacterial flora in saline environments

### Nitrogen cycle

Bacteria may alter some of their biochemical pathways and biochemical activities under conditions of higher salinity. One of these activities is the nitrogen cycle, which is mediated by bacteria and might be affected by drastic extreme saline conditions. The mineralization and immobilization of nitrogen (Bandyopadhyay and Bandyopadhyay 1983), nitrification and ammonification (Wollenweber and Zechmeister-Boltenstern 1989) were decreased in saline soils. The nitrification process was inhibited in soil after addition of sodium chlorate (Azhar et al. 1989) or as a result of soil salinity and drying (Stark and Firestone 1985), which brings about cell dehydration. However, marine or estuarine nitrifying bacteria (e.g. *Nitrosomonas* spp.) were found to be adapted to a wide range (0–30%) of salinity (Macfarlane and Herbert 1984); these bacteria seem to be acclimatized to the fluctuations in salinity in the estuarine environments. However, high rates of denitrification were found in salt marsh ecosystems (Kaplan et al. 1979). The magnitude of effects of soil salinity on the denitrification process is dependent on the type of nitrogen compound ($NO_3^-$-N, $NH_4^+$-N or organic nitrogen) that is present in the soil (El-Shinnawi et al. 1982). Conditions which disturb the nitrogen cycle or that lead to the disappearance of nitrate ($NO_3^-$) from saline soil through the denitrification process might affect soil fertility and the existence of plants and microorganisms in these habitats.

Saline habitats are N-poor (Sprent and Sprent 1990); therefore the N input is very important in these environments. An increasing supply of N dramatically increases production and the standing crop of saline habitats (Valiela et al. 1976). One of the sources of N input in saline habitats is $N_2$ fixation (Teal et al. 1979; Dicker and Smith

1980; Casselman et al. 1981; Whiting and Morris 1986). Higher rates of $N_2$ fixation in saline soils, compared to non-saline soils and agricultural soils, were reported (Wollenweber and Zechmeister-Boltenstern 1989). The low oxygen tension in saline soils may favour the process of $N_2$ fixation, but the diffusion of gases may be impaired at a higher density and water regime in saline soil, an effect which might reduce $N_2$ fixation (Rice and Paul 1971).

In saline environments, e.g. salt marsh sediments, higher levels of ammonia and nitrate were found, which are usually utilized by plants growing in these habitats (Dicker and Smith 1980; Lee et al. 1986); however, when ammonia and nitrate were present at relatively high levels in saline environments, $N_2$ fixation was found to be partly inhibited (Dicker and Smith 1980; Yoch and Whiting 1986). The occurrence of ammonia or nitrates in saline soils, however, is of vital importance to the saline soil bacteria, since ammonia and nitrates are assimilated to form amino acids (e.g. proline, glutamine) and their derivatives. Some amino acids were found to be essential osmolytes for adaptation of bacteria to the saline environments (Galinski and Trüper 1982; Imhoff and Rodriguez-Valera 1984; Weisser and Trüper 1985; Imhoff 1986). Other saline soil bacteria changed the synthesis pattern of protein under salt stress, and protein synthesis of *Vibrio costicola* was inhibited when the intracellular salinity exceeded 0.5 *M* NaCl (Wydro et al. 1977; Kamekura and Kushner 1984). The total protein content of some bacteria from saline soil was reduced by about 10% when the medium salinity was about 10–15% NaCl (Zahran et al., unpublished). The bacteria, which live under extreme salt conditions, temperature and pH, exhibit extensive modification in the content and structure of protein and lipid composition (Vreeland et al. 1983, 1984).

Several $N_2$-fixing bacterial species, which were isolated from saline environments, have been characterized (Nedwell and Abdul-Aziz 1980; McClung et al. 1983). Nitrogen fixation and $N_2$-ase activity were detected and estimated in salt marsh sediments (Wollenweber and Zechmeister-Boltenstern 1989); which are often attributed to sulphate-reducing and -fermenting bacteria (Gandy and Yoch 1988). Nitrogen fixation in association with the salt marsh grass *Spartina alterniflora* has been reported (Whiting et al. 1986). Although a large number of strains which belong to $N_2$-fixing species of bacteria and cyanobacteria have been identified in salt marshes (Paerl et al. 1981), few of them have been shown to fix nitrogen in pure culture (Sprent and Sprent 1990). Strains of $N_2$-fixing Gram-negative bacteria and nitrogen-fixing *Bacillus* were isolated from saline lands of Egypt (Zahran et al. 1995), and about 75% of these bacteria have shown acetylene reduction in pure culture at 5% NaCl. The diazotrophic and salt-tolerant bacterium *Azotobacter* has been isolated from non-saline habitats (Ibrahim 1974; Mahmoud et al. 1978; El-Shinnawai and Frankenberger 1988), which showed either decreasing $N_2$ fixation rates with increasing salinity (>10% NaCl) or optimum fixation at low salinity (5–10% NaCl). Salt-tolerant *Azotobacter* was also isolated from saline soils (Dicker and Smith 1981; Wollenweber and Zech-

meister-Boltenstern 1989). These bacteria were able to fix $N_2$ at higher levels of salt (15–25% NaCl) and at pH 9.

## Mineralization (breakdown) of macromolecules

The saline environments are colonized by a variety of bacterial populations which might have a role in the chemical breakdown of certain macromolecules. The bacteria may have a role in the chemical breakdown of remains from halophytic plants such as mangroves, which might exist in saline environments. Several species, e.g. *Juncas acutus* and *Cyperus lavigatus*, usually colonize the soil adjacent to salt marshes (Zahran and Willis 1992). Degradation of organic matter in the sediments was attributed to bacterial action (Lowe et al. 1993); a bacterium involved in the degradation of the salt marsh grass *Spartina alterniflora* was isolated from a saline soil (Andrykovitch and Marx 1988). Microorganisms are the primary degraders of lignocellulose (a macromolecular complex of the aromatic heteropolymer lignin and the polysaccharides cellulose and hemicellulose), which is a constituent of detritus of halophytic plants such as *Spartina alterniflora* in salt marsh sediments (Benner et al. 1984 a, b; Bolobova et al. 1992). Some bacteria of the genus *Bacillus*, isolated from salt-affected soils and salt marsh soils of Egypt (Zahran et al. 1992; Zahran et al., unpublished), and *Halocella cellulolytica* (Bolobova et al. 1992) have shown cellulolytic or amylolytic activity under saline conditions. Members of the genera *Staphylococcus* and *Micrococcus*, and other non-identified Gram-positive and Gram-negative bacteria from saline soils of Egypt, have also shown cellulolytic and amylolytic activity under salt stress. In contrast, the pectinolytic bacteria are rare inhabitants in the saline soils, representing only a very small fraction (>1%) of the total bacterial isolates from saline soil of Egypt (Zahran et al., unpublished). The bacteria indigenous in the saline soils usually produce degrading enzymes at higher levels of salinity (up to 10% NaCl), but this level is lower than the salt level necessary for growth. For example, the optimal salt level for growth of *Halocella cellulolytica* was about 2.6 *M* NaCl, whereas the optimal salinity for cellulase activity was about 0.5 *M* NaCl (Bolobova et al. 1992). The production and activity of enzymes (e.g. degrading enzymes) from the saline soil bacteria have salt requirements greater than those of corresponding enzymes from non-saline bacteria (Rengpipat et al. 1988b). Nevertheless, the production of these enzymes is not only determined by salt level, but is also affected by the growth phase (Stock et al. 1989).

## Activities against microorganisms

Microorganisms are highly efficient in their ability to produce many kinds of bioactive compounds. A large number of bioactive metabolites (e.g. antibiotics) have been shown to be produced by various types of bacteria and actinomycetes (Omura and Tanaka 1986). Screening of bacteria

from saline habitats or those grown under extreme cultural conditions is one possible way to discover new antibiotics. Some new antibiotics were produced by certain bacteria when a medium with high alkalinity was used (Sato et al. 1983). The marine bacterium *Streptomyces tenjimariensis*, a producer of an aminoglycoside istamycin, successfully grew at about 5–7% NaCl in culture medium (Hotta et al. 1980) and the production of the antibiotic aplasmomycin was best in the presence of 1–3% NaCl (Okami et al. 1976). In addition, inorganic salts (trace or essential elements) are usually added to synthetic media and to natural (beef-extract-containing) media for the production of antibiotics (Iwai and Omura 1981). In a recent research study (Zahran et al., unpublished), bacteria from saline soils of Egypt were screened for their activity (ability to limit or inhibit growth) against other microorganisms. The preliminary results indicated that about 50% of the saline-soil isolates exhibited antimicrobial activity against *Staphylococcus aureus, E. coli* and *Serratia* sp., the activity being detected under non-saline conditions and at moderate salinity (5% NaCl). These preliminary results have encouraged further research work to identify the metabolites produced by saline-soil bacteria.

## Practical significance of the bacterial flora in saline environments

The description of bacteria in saline habitats suggests an important role of these organisms in nature (Ramos-Cormenzana 1991). Nevertheless, an understanding of the ecology of the saline soil bacteria remains extremely limited and data on bacterial community sizes in saline and hypersaline environments are scarce (Oren 1991). The bacteria that prefer unusual extreme conditions to normal conditions might offer an important research tool for investigating the relationships and interactions between environmental factors and microbial life (Gould and Corry 1980). The bacteria of saline environments may be considered models for biological salt tolerance (Lanyi 1979). These organisms have evolved in saline environments and are able to overcome the deleterious effects of salts up to saturating concentrations. Their intracellular components, enzymes, ribosomes, membranes, etc., have been modified in a variety of ways which provide the physical-chemical basis of the salt tolerance. Further, these bacteria which inhabit the extreme saline habitats have been considered useful objects for ecological and evolutionary studies, and the mechanisms which allow the organisms to adapt to extreme saline environments are one of the most interesting subjects for microbiologists (Horikoshi and Akiba 1982). The protective mechanisms evolved in bacteria in saline environments may have commercial significance (Galinski and Tindall 1982). Scientific interest in the extremophilic microorganisms, especially halophilic bacteria, has recently increased (Lowe et al. 1993). One reason for this interest is the need to understand the biochemical mechanisms involved under these extreme conditions because of possible biotechnological use of enzymes and molecules from such organisms (Ollivier et al. 1994). It has been suggested that the bacteria of saline habitats might be able to benefit from the agricultural wastes and produce other useful microbial products, e.g. biopolymers (Ramos-Cormenzana 1991), which are economically important (Gessey 1982). The ability of halophilic bacteria (Fernandez-Castillo et al. 1986) and a salt-tolerant *Rhizobium* sp. (Natarajan et al. 1995) to produce considerable amounts of poly-B-hydroxy butyrate and extracellular polysaccharides (Anton et al. 1988) was also reported. The production of organic acids (e.g. acetic, lactic and propionic) from organic industrial wastes by anaerobic salt-tolerant bacteria would have biotechnological significance (Lowe et al. 1993). Organic industrial wastes are naturally high in salts; these salt conditions may favour the growth of salt-tolerant bacteria and stimulate production of these acids. Further, it has been suggested (Denariaz et al. 1989) that the halophilic nitrifying bacteria be used for removal of salinity and nitrate from waste water for recycling. The purified water could be used for irrigation of plants grown in arid soils. One of the problems with saline and hypersaline lands such as salt-affected soils is the relatively low microbial activity in these soils, which affects vegetal and crop productivity. Therefore, the isolation of active bacteria from saline soil will allow the use of these bacteria in the reclamation of saline soils. The $N_2$-fixing bacteria isolated from saline soil (Zahran et al. 1995) could be good candidates for use to improve the fertility of reclaimed arid and saline soils.

## Conclusions

Saline environments have been largely ignored. Saline lands, for example, are usually considered as abandoned lands, which are no longer producing and will never be explored. However, the accumulated results of recent microbiological research have revealed the economic importance of these saline habitats, which are colonized by selected species from various recognized bacterial groups contributing to the economy of these habitats. The bacteria which flourish in these environments may retain the potential to express various types of activity under extreme conditions. The identification and the taxonomic study of bacteria in saline environments usually takes priority and has received much attention, while research work concerning the activity of these bacteria is, to a large extent, rare. Therefore further research work on the enzymatic activity of bacteria (aerobic and anaerobic) of saline environments and their activity against microorganisms is needed. Nitrogen fixation activity is very important for saline habitats, and until now research on this process has received little attention. Few bacteria isolated from saline soils have proved to be nitrogen fixers. It is expected that these nitrogen fixers colonize the rhizosphere of halophilic and salt-tolerant plants because of the presence of plant exudates, and they may be associated with the roots of these plants or live intracellularly in symbiosis with the host plant. What is the role of bacterial genes in controlling the osmo-

tic adjustment and tolerance of nitrogen-fixing bacteria under saline conditions? This is a new research field which needs to be develop since it has been recently reported (Smith and Smith 1994a) that the accumulation of organic solutes in cells of *Rhizobium meliloti* under osmotic stress may require genetic induction.

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