

OSMOREGULATION IN HALOPHILIC BACTERIA

Hans Jörg Kunte

Institute for Microbiology & Biotechnology, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany

Keywords: chemical potential of water, archaea, bacteria, halophiles, compatible solute, ectoine, glycine–betaine, trehalose, osmoregulation, osmoadaptation, salt-in-cytoplasm mechanism, organic-osmolyte mechanism, osmosensing, osmoregulated transporter, mechanosensitive channel, potassium, stress protection

Contents

1. Introduction
 2. Mechanisms of Osmoadaptation in Prokaryotes
 - 2.1. Thermodynamic Principles Underlying Osmotic Stress
 - 2.2. Salt-in-Cytoplasm Mechanism
 - 2.3. Organic-Osmolyte Mechanism
 - 2.3.1. Stress Protection by Compatible Solutes
 - 2.3.2. Compatible Solutes of Halophiles and Their Synthetic Pathways
 - 2.3.3. Compatible Solute Transport and Osmosensing
 3. Primary Response of the Halophilic Cell to Fluctuation in External Salinity
 - 3.1. Response to Sudden Decrease in External Salinity (Osmotic Downshock)
 - 3.2. Primary Response to Sudden Increase in Salinity (Osmotic Upshock)
 - 3.2.1. Osmoregulatory Response of Nonhalophiles to Osmotic Upshock
 - 3.2.2. Osmoregulatory Response of Halophilic Bacteria to Osmotic Upshocks
- Acknowledgements
Glossary
Bibliography
Biographical Sketch

Summary

Halophiles, which require more than 0.5 mol L^{-1} NaCl for optimal growth, have developed two different basic mechanisms of osmoregulatory solute accumulation to cope with ionic strength and the considerable water stress: the salt-in-cytoplasm mechanism and the organic-osmolyte mechanism. The salt-in-cytoplasm mechanism is employed by extremely halophilic archaea and by bacterial fermenting and acetogenic anaerobes and sulfate reducing bacteria. The organic-osmolyte strategy is widespread in bacteria and also present in methanogenic archaea. To achieve an osmotic equilibrium, the latter group accumulates nonionic, highly water-soluble organic compounds called compatible solutes. Both mechanisms allow halophilic microorganisms to proliferate even in saturated salt solutions of varying salt composition, but the osmolyte strategy allows a more flexible adaptation to environments with changing salt concentrations. This paper discusses the characteristics of compatible solutes and their contribution to stress protection, as well as the accumulation of osmolytes by *de novo* synthesis and osmoregulatory transport from the medium. The adaptation of halophiles using the osmolyte mechanism to sudden fluctuations in salinity of the surrounding environment

is also considered. The focus will be on the export of compatible solutes via mechanosensitive channels to cope with stress caused by dilution of the medium (e.g., by rainfall) and the role of inorganic ions in the adaptation to sudden increases in salinity.

1. Introduction

Concentrated salt solutions, like salt lakes, coastal lagoons, or human-made salterns, inhabited by only a few forms of higher life, are dominated by prokaryotic microorganisms. Global salt deposits show that evaporation of marine salt water and the development of hypersaline habitats has been an ongoing process for millions of years, providing ample time for the evolution of specialized halophilic bacteria and archaea. Halophilic prokaryotes, which require more than 0.5 mol L^{-1} NaCl, have developed two different basic mechanisms of osmoregulatory solute accumulation to cope with ionic strength and the considerable water stress. These mechanisms allow halophiles to proliferate in high saline solutions of varying salt composition, even at saturated concentration. High saline waters originate either by seawater condensation (thalassohaline) or by evaporation of inland surface water (athalassohaline). The salt composition of thalassohaline waters resembles that of seawater, with NaCl as the main constituent. Athalassohaline lakes can differ in their ion composition from seawater-derived lakes. Some athalassohaline waters have a very high concentration of divalent cations (e.g., the Dead Sea with Mg^{2+} , not Na^+ , as the main cation), while others are free of magnesium and calcium due to the presence of high levels of carbonate. Increased carbonate concentrations lead to the formation of soda lakes, which have pH-values well above 10 (e.g., the Wadi Natrun in Egypt). Microflora have been found in all of the above types of saline waters, indicating that halophilic microorganisms tolerate high salinity and can adapt to additional different stressors like high pH or extreme temperatures.

Halophilic bacteria and archaea are not only found to exist in concentrated brines of different mineral composition, but can even survive entrapment in salt rock. This was recently proven by the isolation of a viable halophilic bacterium related to the genus *Bacillus* from subsurface salt deposits of Permian age (~250 million years). This finding brought halophiles into focus in the discipline of astrobiology; if halophilic bacteria can remain in viable states for long periods of time, it is reasonable to consider the existence of life in similar extraterrestrial environments, such as Mars.

The ability of halophilic microorganisms to withstand extreme stress factors is not only fascinating from a pure scientific viewpoint, but also from the viewpoint of biotechnology. For applied sciences, it is of interest to understand how halophilic bacteria can stabilize biomolecules when subjected to harsh conditions. As shown below, organic osmolytes, which some halophiles synthesize and accumulate to establish an osmotic equilibrium, were determined to also act as stabilizers of biological structures. Research in this field since the early 1990s led to the development of novel biotechnological processes and the exploitation of halophilic bacteria for the production of stabilizing osmolytes, which are now marketed successfully.

The objective of this review is to describe the osmoregulatory mechanisms in halophilic bacteria, which allow them to survive in extreme environments and which brought these organisms into the focus of different research areas. To understand the phenotypic responses of bacteria to high salinity, it is important to recall the principles causing osmotic stress. Starting with the thermodynamic principles underlying osmotic stress, this review will introduce the different strategies of osmotic adaptation found in prokaryotes. This will be followed by a discussion of the osmoregulatory mechanisms used by halophilic bacteria to adapt to constantly high salinity (chronic stress), compared to osmoregulatory mechanisms necessary to cope with fluctuations in salt concentration (acute stress).

2. Mechanisms of Osmoadaptation in Prokaryotes

2.1. Thermodynamic Principles Underlying Osmotic Stress

Adding a solute like NaCl to water will lead to changes in the characteristics of the solvent water's freezing and boiling points as well as vapor and osmotic pressures. These changes are caused by the decrease of the water's chemical potential μ_w (in J), which can be expressed as:

$$\Delta\mu_w = \Delta H_w - T\Delta S_w \quad (1)$$

where ΔH (in J) is the change in enthalpy (the heat of reaction), T (in K) is temperature, and ΔS (in J K^{-1}) is the degree of randomness (change in entropy).

According to Sweeney and Beuchat, the second term of Eq. 1 ($T\Delta S_w$) is dominating, and the decrease of the chemical potential largely depends on the change in entropy of the water. This is explained by the interference of salt with the ordered water structure, thereby increasing the randomness of the solvent molecules. The entropy of the solvent, ΔS_w , will therefore be positive, resulting in a reduction of the chemical potential for water, μ_w . An unadapted organism exposed to a saline environment must cope with a higher chemical potential in its cytoplasmic water than in the water of the surrounding environment. Water always flows from a high to low chemical potential until the potential gradient is abolished. Thus, the cytoplasm, which is surrounded by a cytoplasmic membrane freely permeable to water, will lose its cytoplasmic water, resulting in cell shrinkage. The reduction in cell volume is mainly caused by the loss of free water (~80% of total water in a fully hydrated cell), while the bound water level remains unchanged. This results in the cessation of growth, possibly due to molecular crowding, and thus reduced diffusion rates of proteins and metabolites. In order to gain sufficient free water and to maintain an osmotic equilibrium across the membrane, the cell has to reduce the chemical potential of the cytoplasmic free water. Two principle mechanisms have evolved to lower the chemical potential of cell water, allowing an osmotic adaptation of microorganisms: the salt-in-cytoplasm mechanism and the organic-osmolyte mechanism.

- Salt-in-cytoplasm strategy: Organisms following this strategy adapt the interior protein chemistry of the cell to high salt concentration. The thermodynamic

adjustment of the cell can be achieved by raising the salt concentration in the cytoplasm to a similar concentration as in the surrounding environment.

- Organic-osmolyte strategy: In this strategy, organisms keep the cytoplasm, to a large extent, free of NaCl and the design of the cell's interior remains basically unchanged. The chemical potential of the cell water is mainly reduced by an accumulation of uncharged, or zwitterionic, highly water-soluble, organic solutes.

2.2. Salt-in-Cytoplasm Mechanism

The “salt-in-cytoplasm mechanism,” first discovered in halobacteria, is considered the typical archaeal strategy of osmoadaptation. Fermenting bacteria, bacterial acetogenic anaerobes (*Haloanaerobium*, *Halobacteroides*, *Sporohalobacter*, *Acetohalobium*), and sulfate reducers are now known to employ this strategy as well.

Despite the abundance of NaCl in the typical haloarchaeal environment, halophilic archaea keep the cytoplasm relatively free of sodium. Instead, potassium accumulates in the cell (as proven for *Haloferax volcanii* through an energy-dependent potassium uptake system) and, together with its counterion Cl^- , K^+ can be found in molar concentrations in the cytoplasm. Because the K^+ concentration inside the cell is 100 times higher than in the surrounding environment, a part of the proton motive force must be used to maintain the ion gradient. In this energetic respect, the situation in halophilic anaerobic bacteria is thought to be different; there is evidence that these organisms invest as little as possible in the maintenance of ion gradients. Measurements of the ion composition of exponentially growing cells of *Haloanaerobium praevalens* show that K^+ is the dominating cation, but that Na^+ levels are also relatively high. Cells entering the stationary phase eventually replace K^+ with Na^+ . Analysis of *Haloanaerobium acetoethylicum* even suggests that Na^+ could be the main cation in stationary cells as well as in exponentially growing cells.

The effect of the accumulation of potassium and/or sodium in the cytoplasm is that the cytoplasm is exposed to an increased ionic strength. To adapt the enzymatic machinery to an ionic cytoplasm, proteins of halophilic anaerobic bacteria and halophilic archaea contain more acidic amino acids than basic residues. This leads to a predominance of charged amino acids on the surface of enzymes and ribosomes, which is thought to stabilize the hydration shell of the molecule when in highly ionic surroundings. In low-saline environments, the excess of negatively charged ions will destabilize the molecule's structure due to repulsion when the shielding cations are removed. This mechanism explains the fact that organisms employing the salt-in-cytoplasm strategy display a relatively narrow range of adaptation and that their growth is restricted to highly saline environments. However, in habitats with saturated salt concentrations, halophilic archaea out-compete compatible solute producers, establishing users of the salt-in-cytoplasm mechanism as *extreme* halophiles.

2.3. Organic-Osmolyte Mechanism

The organic-osmolyte mechanism is widespread among bacteria and eukaryotes and is also present in some methanogenic archaea. In response to an osmotic stress, these organisms mainly accumulate organic compounds like sugars, polyols, amino acids,

and/or amino acid derivatives, either by *de novo* synthesis or by uptake from the surrounding environment. These nonionic, highly water-soluble compounds do not disturb the metabolism, even at high cytoplasmic concentrations, and are thus aptly named compatible solutes. Halophilic cells using compatible solutes can basically preserve the same enzymatic machinery as nonhalophiles, needing only minor adjustments in their interior proteins (i.e., ribosomal proteins), which are slightly more acidic than the cytoplasmic proteins in *Escherichia coli*. Halophiles employing the organic-osmolyte mechanism are more flexible than organisms employing the salt-in-cytoplasm strategy because, even though they display wide salt tolerance, they can also grow in low salt environments. If the solutes must be synthesized *de novo*, however, this flexible mode of osmoadaptation is a more energy-demanding process than the accumulation of inorganic ions. It was calculated that the synthesis of one compatible solute like ectoine or glycine–betaine, which are commonly found in halophiles as main osmolytes, requires about 40 ATP-equivalents. In comparison, organisms using salt-in-cytoplasm mechanism are assumed to invest as little as one molecule of ATP to transport two K^+ and two Cl^- from the environment. Organisms of the organic-osmolyte strategy can also switch to the energetically favorable mode of solute accumulation by transporting organic osmolytes into the cytoplasm as outlined below.

2.3.1. Stress Protection by Compatible Solutes

In addition to their function of maintaining an osmotic equilibrium across the cell membrane, compatible solutes are effective stabilizers of proteins and even whole cells. They can act as protectors against heat, desiccation, freezing and thawing, and denaturants such as urea and salt. The reason why these organic compounds are compatible with the metabolism and can even act as stabilizers of labile biological structures is explained at the molecular level by the preferential exclusion model. According to this theory, compatible solutes are absent from protein surfaces due to the dense structural water bound to the protein.

Compatible solutes show a preference for the less dense free-water fraction in the cytoplasmic surroundings. They stabilize the two water fractions by fitting into the lattice of the free water and allowing for the formation of hydration clusters. As a consequence, unfolding and denaturation of proteins becomes thermodynamically unfavorable (reinforcement of hydrophobic effect). This explains why organisms adapted to other low-water-potential environments take advantage of the beneficial properties of compatible solutes. It is not surprising that cyanobacterial species found in deserts accumulate the compatible solute trehalose to compensate for the deleterious effects of desiccation.

-
-
-

TO ACCESS ALL THE 15 PAGES OF THIS CHAPTER,
Visit: <http://www.eolss.net/Eolss-sampleAllChapter.aspx>

Bibliography

Brown A.D. (1980). *Microbial Water Stress Physiology—Principles and Perspectives*. Chichester: John Wiley and Sons. 313pp. [This book describes the thermodynamic fundamentals underlying osmotic stress.]

Csonka L.N. and Epstein W. (1996). Osmoregulation. *Escherichia coli and Salmonella*, 2nd edition (ed. F.C. Neidhardt), 1210 pp. Washington: ASM Press. [This chapter presents an overview of the osmoregulatory mechanisms in enteric bacteria.]

Galinski E.A. (1995). Osmoadaptation in Bacteria. *Advances in Microbial Physiology*, Vol. 37, 273 pp. London: Academic Press. [Most comprehensive overview on osmoregulatory mechanisms in halophilic bacteria, emphasizing characteristics of compatible solutes found in bacteria, compatible solute synthesis, and regulation of their biosynthetic pathways.]

Grammann K., Volke A., and Kunte H.J. (2002). A new type of osmoregulated transporter identified in halophilic bacteria: TRAP-transporter TeaABC mediates the uptake of ectoine and hydroxyectoine in *Halomonas elongata* DSM 2581^T. *Journal of Bacteriology*. **184**, 3078-3085. [This paper describes the first osmoregulated transporter found in halophilic bacteria.]

Louis P. and Galinski E.A. (1997). Characterization of genes for the biosynthesis of the compatible solute ectoine from *Marinococcus halophilus* and osmoregulated expression in *Escherichia coli*. *Microbiology* **143**, 1141–1149. [This article describes the first characterization of the genes responsible for the synthesis of ectoine, the typical compatible solute of chemoheterotrophic, halophilic bacteria.]

Oren A. (1999). Bioenergetic aspects of halophilism. *Microbiology and Molecular Biology Reviews* **63**(2), 334–348. [In this article, the energetic costs of the two different modes of osmoadaptation are compared and the preference of microorganisms for certain compatible solutes is explained on the basis of their energetic costs.]

Racher K.I., Voegelé R.T., Marshall E.V., Culham D.E., Wood J.M., Jung H., Bacon M., Cairns M.T., Ferguson S.M., Liang W.-J., Henderson P.J.F., White G., and Hallett F.R. (1999). Purification and reconstitution of an osmosensor: transporter ProP of *Escherichia coli* senses and responds to osmotic shifts. *Biochemistry* **38**, 1676–1684. [This research article describes the first successful reconstitution of an osmoregulated transporter in membrane vesicles.]

Stumpe S., Schlösser A., Schleyer M., and Bakker E.P. (1996). K⁺ circulation across the prokaryotic cell membrane: K⁺-uptake systems, *Handbook of Biological Physics*, Vol. 2 (eds. W.N. Konings, H.R. Kaback, J.S. Lolkema), North-Holland: Elsevier Science. 473 pp. [This chapter outlines the features of osmoregulated potassium transport systems found in *Escherichia coli*.]

Ventosa A., Nieto J.J., and Oren A. (1998). Biology of moderately halophilic bacteria. *Microbiology and Molecular Biology Reviews* **62**(2), 504–544. [This review gives an overview on the ecology, physiology, and biotechnology of chemoheterotrophic, halophilic bacteria.]

Wood J.M. (1999). Osmosensing by bacteria: signals and membrane-based sensors. *Microbiology and Molecular Biology Reviews* **63**(1), 230–262. [This article explains the properties of water–macromolecule interactions necessary to understand osmosensing and (putative) osmosensors and the changes that those sensors can detect.]

Biographical Sketch

Upon receiving his diploma in microbiology, with emphasis on biochemistry and genetics, **Hans Jörg Kunte** began his PhD studies in osmoadaptation of halophilic bacteria in the laboratory of Professor Erwin Galinski, at the University of Bonn, Germany. These studies were completed in 1995. Dr. Kunte's postdoctoral studies took him to the University of Guelph, Canada, where he worked in the laboratory of Dr. Janet Wood on the reconstitution and regulation of transport proteins responsible for compatible solute uptake in enteric bacteria. He then returned to the University of Bonn to take over the group working on the biotechnology of halophilic bacteria. Currently the group, which includes three doctoral

candidates, two masters candidates, and two technicians, is working on osmoregulated uptake of compatible solutes in halophilic bacteria. The goal is to apply any regulatory mechanisms to the field of biotechnology. Dr. Kunte has recently presented his findings at a conference in Hamburg on extremophiles and at the annual meeting of microbiologists in Oldenburg.

UNESCO – EOLSS
SAMPLE CHAPTERS