HALOPHILY (HALOPHILISM AND HALOPHILIC MICROORGANISMS)

Ventosa A. and **Arahal D. R.**
Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, Spain

Keywords: Halophilic microorganisms, salt, NaCl, hypersaline, biodiversity, physico-chemistry, extreme environment, saline lakes, salterns, saline soils, osmotic stress, compatible solutes, biotechnology

Contents

Summary

Hypersaline environments are characterized by their high concentration of salts, especially NaCl. Besides this, they may have other characteristics, that overall make the conditions for living very difficult, and for that reason they are considered as extreme environments. Among these other features are their low dissolved oxygen concentration, high or low temperatures, high pH values, solar irradiation, etc. They are represented by terrestrial as well as by two different aquatic habitats, named thalassohaline and athalassohaline, depending upon whether their relative salt proportions are similar or not to that of seawater. The most detailed studies of hypersaline habitats are from saline lakes (Dead Sea, Great Salt Lake, Wadi Natrun, etc.), marine salterns (which are used for the production of marine salt by evaporation of seawater), and saline soils.

Organisms that inhabit these saline environments are called halophiles. The three groups of organisms that are normal inhabitants of these habitats are: i) halobacteria, or extremely halophilic aerobic archaea; members of the family Halobacteriaceae, which includes several species grouped in 15 different genera; ii) moderately halophilic bacteria, that grow best at lower salinities than halobacteria and are predominant organisms in environments with intermediate salinities. They are represented by a variety of species and genera belonging to the methanogens and to different

phylogenetic branches of the bacteria (Proteobacteria, High- and Low G+C Gram-positive bacteria, Spirochetes, Cytophaga-Flexibacter-Bacteroides branch, Cyanobacteria, etc.); and iii) some eukaryotic organisms, such as the brine shrimp *Artemia salina* or the blue green algae *Dunaliella.*

In this introductory article, different aspects of the phylogeny, taxonomy, biodiversity, physiology and biotechnological applications of the halophilic microorganisms are discussed.

1. Introduction

In the history of humankind, salt has been a valuable resource, especially in places where it is not naturally abundant or easy to import. It is necessary as a nutrient, but it was also used as a preservative against microbial deterioration, a long time before it was known what bacteria affecting fish, meat, hides and other goods were. This practice led to an empirical association between high concentration of salt and the absence of life. The concept had, however, some occasional exceptions, in which a reddening and spoilage of salt-preserved goods could be seen. By the end of the 19th century, it was already accepted that this phenomenon was due to microbial growth occurring at salt concentrations inhibitory for most other organisms.

What about habitats with a natural high salt concentration? The presence of life is not that hard to detect, only some ability to observe and question is required. Looking at a solar pan anywhere in the world, at the “Great Salt Lake”, at the “Solar de Atacama” or at any other hypersaline environment, it is possible to detect – when the conditions are favorable – a red coloration (sometimes preceded by green) due to microbial growth. If the environment is aqueous, then the coloration can be very intense, indicating the presence of a dense community of microorganisms.

The next questions are ‘what are the causative agents of the reddening?’ and ‘is this reddening all that thrives in these habitats?’ The classical answer to these questions would be that the reddening is caused by archaea, which are the major (or only) inhabitants of hypersaline habitats at the highest salinities. At lower saline concentrations, a more significant contribution of bacteria and eukarya can be expected. However, this view has changed recently, since molecular techniques have shown that, at least in some hypersaline environments, archaea are not as predominant in the upper range of salinities as it was thought. Besides, the finding of *Salinibacter ruber*, a red-pigmented extremely halophilic bacterium, has proved that even the reddening in hypersaline habitats is no longer a trait exclusive of halophilic archaea. Certainly, we have a sound knowledge of life under hypersaline conditions, but we are far from knowing all its secrets.

2. Halophilism: Concept and Classifications

By simply using its etymological origin, halophilism can be defined as “love for salts” or in a more explanatory way and applied to living organisms, it refers to a physiological need for salts above common values. As salt is present in the chemical composition of any organism and is an essential nutrient, it is important to stress ‘above common' since otherwise any living organism could be considered halophilic to a certain extent. Indeed, only a reduced group of organisms (compared to the total biological diversity) is termed as halophilic, as will be discussed later. In this sense, those halophilic organisms that inhabit hypersaline habitats are considered extremophiles, meaning that they develop in environmental conditions that would be too harsh to most organisms. The definition of extremophilic is not deprived from an anthropocentric perspective and thus, it considers situations in which a certain environmental variable is significantly deviated from what we may consider normal (pH close to neutrality, room temperature, low salinity, high nutrient availability and so on). As these features are not necessarily exclusive, it is possible to find environments, and organisms that thrive in them, that are haloalkaliphilic, psychrophilic and halophilic, barophilic and halophilic, etc.

So far, salts have been referred to in a general sense and no values have been mentioned. A scientific approach deserves more accuracy, although in this subject it is not an easy task because the boundaries are rather imprecise. If we look in a dictionary for a definition of 'salt' as a noun, the first entry we find is very likely to look like this: 'crystalline compound that consists of sodium chloride (NaCl), is abundant in nature, and is used especially to season or preserve food or in industry - synonym common salt'. Other substances resembling common salt (by its ionic nature and so on) can also be termed as salt, therefore when used in plural, salts, we refer to a mineral or saline mixture, as for instance the salts of the seas. Indeed, the ionic composition of hypersaline and saline environments consists of a mixture of salts, not just NaCl. However, because the majority of these niches are thalassohaline and in them NaCl is by far the major component, it is very usual to leave the other ions aside from calculations. Thus, we can say that a brine sample from a saltern pond containing 24 % (weight/volume) salts has 4.1 M NaCl (taking a molecular weight of 58.5 grams per mole). The error derived from excluding other ions is minimal and from the biological point of view is meaningless. Therefore, it is an acceptable assumption.

Some aspects of the terminology have been clarified, but we still need to define the limits employed to delineate halophily. Among the many definitions given by different authors, the most accepted is shown below, and considers the following categories of microorganisms according to their response to salts:

Extreme halophiles	Grow optimally above 15 % salts
Moderate halophiles	Optimal growth is within the range 3-15 % salts
Slight halophiles (marine microorganisms)	Grow best between 1 and 3 % salts
Halotolerants	Optimal growth occurs below 1 % but growth is also observed at higher salt concentrations (15 % or even more)
Non-halophiles	Optimal growth is below 1 % salts

It is important to note, however, that outside of the scientific community specialized on halophiles as extremophilic organisms, all the halo-derived words (i.e. halotolerant, halophilic, haloresistant and so on) are often used to define organisms that exhibit a very mild halophilic behavior that, however, helps to differentiate them from their closest relatives. This misappropriate use of the terminology can bring some confusion, for example if we perform a boolean search on a citations database, we are very likely to get many unwanted entries.

The following sections deal with different aspects, such as phylogeny and taxonomy, ecology, physiology, genetics and biotechnological applications of halophiles. When necessary distinction between the different categories of halophiles will be made, otherwise they will be treated as a hole, or according to their phylogeny.

3. Phylogeny and Taxonomy

Modern prokaryotic taxonomy and phylogeny developed by the mid 1970s, and about ten years later sequence analyses of the small ribosomal RNA subunit was a well established method for assessing phylogeny and inferring taxonomy. The consequence of this has been a tremendous increase in the number of described halophilic species, from 30 in 1980 to almost 200 nowadays. Of course, such an expansion would not have been possible without the effort of many researchers looking for new niches, isolation procedures, cultivation techniques and so on. In the particular case of halophilic archaea, the importance of polar lipids analyses as a contribution for the improvement of the taxonomy of this group has to be mentioned. Indeed, it is one of the key features on the minimal standards recommended for the description of new members within the family *Halobacteriaceae*. In the tree presented in Figure 1, the main phylogenetic groups of archaea, bacteria and eukarya containing moderately or extremely halophilic species have been included.

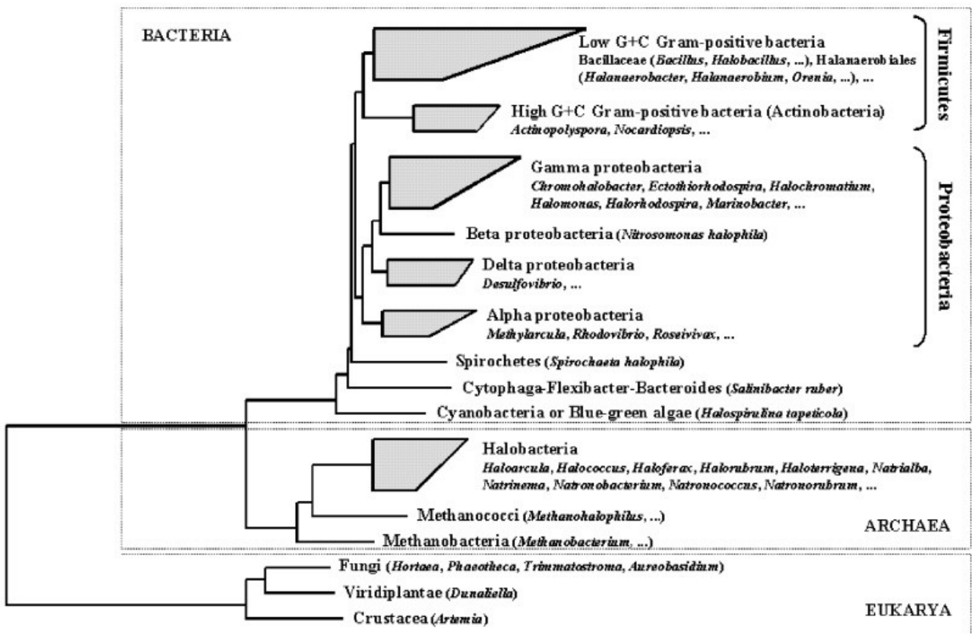

Figure 1. Phylogenetic tree based on the comparison of the 16S rRNA sequences, showing the major groupings of the halophilic members of the Bacteria, Archaea and Eukarya.

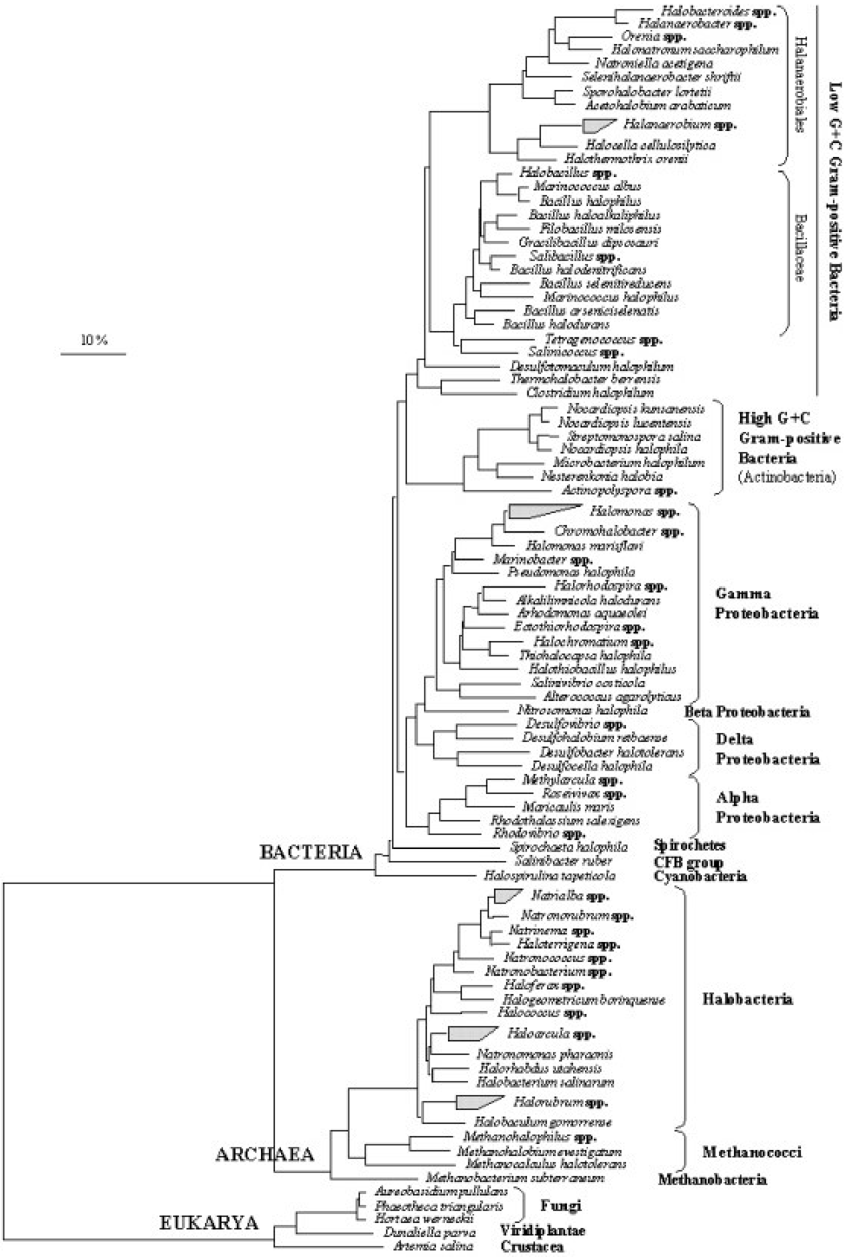

Figure 2. A detailed phylogenetic tree, based on the comparison of the 16S rRNA sequences, in which the relationships among the different species of the three domains (Bacteria, Archaea and Eukarya) are shown.

-
-
-

Bibliography

Arahal D.R., Ludwig W., Schleifer K.H. and Ventosa A. (2002). *Phylogeny of the family* Halomonadaceae *based on 23S and 16S rDNA sequence analyses*. *International Journal of Systematic and Evolutionary Microbiology* **52**, 241-249. [A recent article in which the phylogeny of the halomonads is evaluated, based on the comparison of rRNA sequences].

Grant W.D., Kamekura M., McGenity T.J. and Ventosa A. (2001). *Class III.* Halobacteria *class. nov.* In *Bergey's Manual of Systematic Bacteriology*, 2nd Ed., Vol. 1. Boone, D.R., Castenholz, R.W. and Garrity, G.M. (Eds.). New York: Springer. [The most recent edition of *Bergey's Manual* includes an updated view of the aerobic extremely halophilic archaea (halobacteria)].

Javor B.J. (1989). *Hypersaline Environments. Microbiology and Biogeochemistry*, Berlin: Springer-Verlag. [This book deals with ecological and biogeochemical aspects of different hypersaline environments and halophilic microorganisms].

Madern D., Ebel C. and Zaccai G. (2000). *Halophilic adaptation of enzymes*. *Extremophiles* **4**, 91-98. [This is a review concerning the effects of salt on the activity, solubility and stability of halophilic enzymes].

Margesin R. and Schinner F. (2001). *Potential of halotolerant and halophilic microorganisms for biotechnology*. *Extremophiles* **5**, 73-83. [An updated mini-review describing different applications of halophilic and halotolerant microorganisms].

Oren A. (Ed.) (1999). *Microbiology and Biogeochemistry of Hypersaline Environments*, Boca Raton: CRC Press. [This is a recent book focused on halophilic microorganisms, and includes a collection of excellent contributions presented in a meeting on halophilic microorganisms held in Jerusalem in 1997].

Rodriguez-Valera F. (Ed.) (1988). *Halophilic Bacteria*, 2 Vols. Boca Raton: CRC Press. [This was the first book dedicated exclusively to the review of different aspects of halophilic bacteria and archaea. Although it is not up to date, it can be used as a reference book, and some contributions are still very useful].

Ventosa A. (Ed.) (in press). *Halophilic Microorganisms*. Berlin: Springer. [This book collects most contributions presented in a recent meeting devoted to halophilic microorganisms held in Sevilla in September 2001].

Ventosa A. and Nieto J.J. (1995). *Biotechnological applications and potentialities of halophilic microorganisms*. *World Journal of Microbiology and Biotechnology* **11**, 85-94. [This is a concise mini-review in which the applications as well as potential uses of halophiles are described].

Ventosa A., Nieto J.J. and Oren A. (1998). *Biology of moderately halophilic aerobic bacteria*, *Microbiology and Molecular Biology Reviews* **62**, 504-544. [The most updated review about moderate halophiles, in which different aspects of these halophiles, such as their taxonomy, phylogeny, ecology, physiology, genetics and biotechnological applications are reviewed in detail].

Vreeland R.H. and Hochstein L.I. (Eds.) (1993). *The Biology of Halophilic Bacteria*, Boca Raton, CRC Press. [Several chapters are presented that review different aspects of halophilic microorganisms].

Biographical Sketches

Dr. Antonio Ventosa is a Professor in the Department of Microbiology and Parasitology of the Faculty of Pharmacy of the University of Sevilla, Spain. He served as Dean of the Faculty of Pharmacy for the period 1997-2001. He received his Ph.D. degree in 1981 from the University of Granada (Spain). Dr. Ventosa is an expert on the study of halophilic microorganisms, as well as other extremophilic organisms. He has a great deal of experience in different aspects of halophiles, such as their taxonomy and phylogeny, ecology, physiology, genetics and biotechnological applications. He has published 146 articles in books and scientific journals on halophilic microorganisms and is a member of the editorial boards of the journals *Systematic and Applied Microbiology*, *Extremophiles* and *International Microbiology*. Recently, he prepared six chapters for the second edition of the Bergey's Manual of Systematic Bacteriology (four for vol. 1, published in 2001 and two for vol. 2, in press). In 1991, he gained the "Jaime Ferran" award for Microbiology (by the Spanish Society of Microbiology). He is member of the International Committee on Systematic Bacteriology (ICSB), Chairman of the ICSB-Subcommittee on Taxonomy of Halobacteriaceae and ICSB-Subcommittee on Taxonomy of Halomonadaceae, and a member of the ICSB-Subcommittee on Taxonomy of the genus *Bacillus* and related organisms. He was member of the Executive Board of the World Federation for Culture Collections (1992-1996) and is currently a member of the WFCC-Committee on Capacity Building and Education. He is correspondent member of the Academia Iberoamericana de Farmacia.

Dr. David R. Arahal, born in 1969, completed his Ph.D. in Pharmacy in 1993 at the University of Sevilla (Sevilla, Spain). As a postgraduate researcher, he spent part of the time in foreign institutions: Marine Biological Laboratory, Woods Hole (Massachusetts, USA), Forsyth Dental Center, Boston (Massachusetts, USA) and Technische Universität München (Munich, Germany). At the conclusion of his Ph.D., he continued his experience at the Technische Universität München for two years as a postdoctoral fellow. His professional career has been dedicated to the study of different aspects of halophilic microorganisms, focussing on their phylogeny, taxonomy and ecology, not concentrating his attention to a single microbial group, rather to a variety of them (archaea and bacteria, gram-positive and gram-negative; extreme and moderate halophiles and halotolerant microorganisms). He is very skilled in phylogeny and molecular identification techniques, and his goal is to apply them to hypersaline environments to expand the knowledge on the microbial diversity of such habitats. In 1992, he gained the prize "Aguilar Ceballos" and in 1995 the "Phil H. Presley Scholarship" from Carl Zeiss Inc.