PHYSICO-CHEMICAL CHARACTERISTICS OF HYPERSALINE ENVIRONMENTS AND THEIR BIODIVERSITY

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Summary

Hypersaline environments are extreme habitats where the salinity is high (much higher than that of seawater) and can be divided into two main types, thalassohaline and athalassohaline environments, depending on whether they originated from seawater or not, respectively. Examples of thalassohaline environments that have been studied in detail are the solar salterns (used for the production of salt by evaporation of seawater), some lakes such as the Great Salt Lake in Utah, or saline soils. The athalassohaline environments that have been more extensively studied are the Dead Sea and some alkaline lakes (soda lakes) that are poor in magnesium or calcium ions and abundant in carbonates, resulting in a very alkaline pH (pH 9–11.5). Most ecological studies in hypersaline environments have been based on the traditional methods of isolation and identification of pure cultures. However, recent molecular techniques have improved our knowledge of the microbial diversity of these habitats. The biodiversity is higher than was reported, but obviously lower than that of other nonsaline environments. Besides some eukaryotic representatives (e.g., the brine shrimp *Artemia salina* or the unicellular algae *Dunaliella*), most microbial inhabitants of hypersaline environments

are phototrophic and heterotrophic prokaryotes. In the most hypersaline environments, aerobic Archaea, member of the *Halobacteriales*, are very abundant (e.g., species of the genera *Haloarcula, Haloferax, Halobacterium, Halorubrum*, as well as *Natronobacterium* or *Natronococcus* in the alkaline lakes). However, recent studies have also determined that bacteria represent a high proportion of the inhabitants of these habitats (the bacterial species *Salinibacter ruber*). Saline environments with intermediate salinity are more diverse, and both phototrophic and heterotrophic bacteria are observed. These are represented by a variety of gram-negative and gram-positive aerobic and strictly anaerobic microorganisms that are moderately or extremely halophilic, as well as some halotolerant bacteria. They play an important ecological role in these extreme environments.

1. Introduction

Hypersaline environments attracted the interest of early microbiologists by the second half of the nineteenth century. Occasional spoilage of salt-cured hides and food gave evidence that, although high concentrations of inorganic salts represent too extreme a condition for most microorganisms, other microorganisms can cope and thrive under such circumstances. This motivated other scientists to look for such microorganisms in natural habitats with similar or even higher salt concentrations. Before going into details about the differences that exist among hypersaline environments from the physicochemical point of view, it is appropriate to discuss the terminology and the meaning given to some concepts in the study of halophilism. In the literature about saline and hypersaline environments and halophilic microorganisms, the term “salts” is used to mean a combination of different inorganic anions and cations, of which Cl^- and Na^+ are the most abundant, respectively. So, when a certain salinity is given, such as 20% (mass per volume) or 200 g L^{-1}, and when such values are converted into molar concentrations, the molecular weight of NaCl (sodium chloride, common salt, halite) is taken. Unless we mark some borderlines, it could be said that all living organisms (even humans) are halophilic, since all of them require a certain amount of inorganic salts to live. As a proof of the value and importance of salt, it may be enough to recall that at certain eras and in certain cultures salt was used as a currency (the word “salary” originated from the Latin word “*salaries*,” meaning an extra allowance to buy salts in payment for a service). Nevertheless, the values tolerated or required by most organisms (especially eukaryotes) are generally below 1%, lower than what is generally considered halophilic or halotolerant. The oceans and seas can be considered the most extended habitats in the world, and their salinity, which is fairly constant (around 3.5%), can be tolerated or even required by the organisms that thrive in them. Therefore, we refer to them as slightly halophilic organisms, which can be taken as a synonym of marine organisms.

In a more restricted sense, focusing on the extremophilic side of halophilism, halophiles are organisms that have an optimal growth above 3% salt concentration. When the optimal growth occurs between 3% and 15% salt they are regarded as moderate halophiles and when it occurs above 15% salt and up to halite saturation (34%) they are regarded as extreme halophiles. Of course there are other classifications of halophilism, but this is the most extended one. The concept of halotolerance is not always very easy to match with natural behaviors. It refers to nonhalophilic organisms that can grow at salt concentrations well above 3%, although their optimal growth occurs below this value. Many microorganisms described originally as halotolerant are indeed moderate halophiles, and vice versa. In some cases a different behavior is observed depending on the incubation temperature or the nutrients added. In any case, we have to keep in mind that artificial criteria do not necessarily have to adjust to all possible situations in nature.

Another way of looking at the question of classifying halophilic organisms is to consider the different strategies for coping with a highly saline biotope. Probably the most significant strategy is the acquisition of an internal osmotic pressure equal (or even higher to maintain a turgor) to that on the exterior. In general, microorganisms develop one of two main strategies: the accumulation of nontoxic inorganic ions, which is typical of the halobacteria (and a few examples of bacteria), or the accumulation of organic molecules compatible with their internal metabolic activities (commonly called "compatible solutes") of different chemical nature, which is the usual strategy of moderately halophilic and halotolerant bacteria as well as of eukaryotes. In the first case, microorganisms are sometimes referred to as "true halophiles" since the cytoplasm itself is halophilic, and therefore all enzymes and biological processes must be adapted to such conditions.

If we focus our attention on the environment and not on the biota, we may ask what value of salinity a given environment must have to be considered saline or hypersaline. The answer may vary greatly from one author to the other, but in general "saline" is used to mean anything close to but above sea salt concentration, while "hypersaline" refers to higher values (up to halite saturation). In the case of soils, a content of soluble salts above 0.2% is enough to be considered saline.

Diversity in saline environments is not as restricted as in hypersaline environments. The drop in the microbial diversity of the hypersaline habitats is what makes them to be considered extreme environments. In other words, of all living organisms only a few highly specialized microorganisms are able to tolerate, prefer or even require the conditions existing in extreme environments.

Hypersaline environments can be divided into two main types, thalassohaline and athalassohaline environments, depending on whether they originated from seawater or not, respectively.

2. Thalassohaline Environments

As defined before, thalassohaline environments are saline environments related by origin to the sea, or in a more extended context related by chemical nature to the sea (that is, the proportions of the salts present resemble somehow those found in seawater). We find abundant examples of such environments and each will be discussed separately later.

Chemically, they are characterized by a clear predominance of Cl^- and Na^+ (responsible for 49% and 42% of the total molarity, respectively). Other important ions are Mg^{2+}, SO_4^{2-}, K^+, Ca^{2+}, Br^-, HCO_3^-, and F^-. The average salinity of seawater is 3.5%; when it concentrates (as in a solar saltern) its composition changes due to serial precipitations.

The first salts to precipitate are some carbonates, but in very small amounts. At about 10% salinity, calcium carbonate starts to precipitate. The main precipitation is that of NaCl (halite), which takes place at 34% salinity. The remaining brines must be doubly concentrated to reach the precipitation point of Mg^{2+} and K^{+} salts. But from the microbiological point of view those brines are of little interest since no evidence of life has ever been observed in them.

2.1. Solar Salterns and Coastal Lagoons

These hypersaline habitats are widely distributed and can be found at sea level in arid and semiarid regions, both as naturally occurring or human-made salterns. Among the natural ones, *sabkhas* ("salt-flat" in Arabic) are a good example, but are only occasionally studied. Solar salterns typically consist of several ponds interconnected to form the so-called multipond system. Seawater is pumped or allowed to flow into the first ponds. As a consequence of solar evaporation the concentration of salts increases slightly and the water is moved to the next ponds, where it will concentrate further. Finally, in the last ponds (called crystallizers) common salt is precipitated.

Many authors have focused their attention on these interesting systems, given their great value as models of aquatic environments with increasing salinity. As expected, each pond has a population composition accordingly to its salinity. Below 10% the eukaryotic diversity is richer, and organisms such as protozoa, green algae, diatoms, and larvae can be found. The prokaryotic diversity is also higher below 10% and consists of heterotrophic and phototrophic organisms. At higher salinity, *Dunaliella* spp. (a microscopic green algae) and *Artemia salina* (brine shrimp) are the only eukaryotes detectable. The drop in diversity applies to the prokaryotes as well, yet it is still possible to find heterotrophic Proteobacteria (such as *Halomonas* spp. or *Salinivibrio* spp.), Firmicutes (of the genus *Bacillus* and related genera), and flavobacteria. Phototrophic bacteria (such as some cyanobacteria) are observed at salinities up to but not higher than 16%. Concerning the presence of archaea, halobacteria become more significant and even predominant in the overall diversity at higher salinities. Species of the genera *Haloarcula*, *Haloferax*, *Halorubrum*, and *Halobacterium* (to name the most significant ones) are commonly isolated from solar salterns, even from the crystallizers. It is commonly thought that salterns lack halophilic fungi; however, this is not supported by some studies in which a number of polymorphic black yeasts have been isolated from saltern hypersaline waters (3–30% NaCl). These include species of the genera *Hortaea*, *Phaeotheca*, *Trimmatostroma*, *Aureobasidium*, and *Cladosporium*.

Mediterranean salterns, especially those located along the Spanish seashore, are among the most studied hypersaline environments. The climatic conditions of this region permit the exploitation of salterns for salt production several months of the year. From April to September, the light exposure largely exceeds that of other climates. The water temperature in the ponds can go up to 45 ºC or even higher in summer. The warm temperatures favor the growth and development of some halophilic microorganisms.

2.2. Soils

If it is a dry soil it has very little water available. The availability of this water is further reduced by the presence of salts. For this reason, a soluble salts content exceeding 0.2% in soil is enough to classify it as saline. While the relative humidity of the air in moderate climates is in the range of 50–90%, in desert environments it can be as low as 5%. The problem of desiccation requires specific strategies for survival apart from those derived from a saline stress. These strategies include the synthesis of desiccation-resistant proteins, the accumulation of nonreducing sugars, and the formation of dormant life stages (endospores). Additional stress factors are the temperature shifts between night and day and the scarcity of precipitation (less than 200 mm y^{-1} rainfall in arid regions and less than 25 mm y^{-1} rainfall in hyperarid regions). In light of all this, it is not surprising that the diversity found in such habitats is extremely reduced and is almost limited to some low G+C Firmicutes and some high G+C Actinomycetes. Their abundance is approximately 10^6 CFU g^{-1} (colony-forming unit) of soil in arid regions and one or two orders of magnitude lower in hyperarid regions.

Nonarid hypersaline soils are a special kind of extreme environment where the salt concentration is much more variable (for example, as a consequence of rainfall or due to local variations) than it is in hypersaline waters. At a microbial scale, one cubic centimeter of soil can contain habitats as different as a salt crystal, a piece of debris, or a drop of moisture; the surface will be completely exposed to light and the rest in darkness. Studies conducted on hypersaline soils describe the isolation of moderate halophiles and nonhalophilic bacteria affiliated with different genera of the following taxonomic groups: high G+C gram-positive bacteria, low G+C gram-positive bacteria, gamma-proteobacteria, beta-proteobacteria and flavobacteria. Halobacteria have been also described occasionally.

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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 PhD 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 extensive 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 was given the “Jaime Ferran” award of 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 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 currently is member of the WFCC-Committee on Capacity Building and Education. He is a correspondent member of the Academia Iberoamericana de Farmacia.

David R. Arahal, born in 1969, completed his PhD 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 PhD he continued his experience at the Technische Universität München for two years as a postdoctoral fellow. His professional career has been devoted to the study of different aspects of halophilic microorganisms, focusing on their phylogeny, taxonomy and ecology. He chose not to focus his attention to a single microbial group, but to study a variety of them (archaea and bacteria, gram-positive and gram-negative; extreme and moderate halophilic 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 of the microbial diversity of such habitats. In 1992 he was awarded the "Aguilar Ceballos" prize and in 1995 the "Phil H. Presley Scholarship" from Carl Zeiss Inc.