**Halophilic micro-organisms and their adaptations**

**Life at low water**

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Life, or at least life as we know it, depends on water. Water is the solvent in which the cytoplasmic enzymatic machinery functions. However, life on Earth has adapted to a variety of environments with low water activity. Availability of water can be reduced both by the presence of salts and other solutes in the cells' surroundings ('osmotic water stress') and by drought ('matric water stress'). As biological membranes are permeable to water, intracellular water activity equals that of the outside medium. A water activity of 0.60–0.62 appears to be the lower limit for life. Salt lakes and other hypersaline environments are populated by a diverse world of micro-organisms adapted to life at salt concentrations up to NaCl saturation. The study of those organisms growing at the highest salinities shows that the problem how to cope with low water activity can be solved in different ways.

**Water availability and life**

Many micro-organisms can survive prolonged exposure to dryness. Endospores of *Bacillus* and *Clostridium* are most famous in this respect, and there are reported cases of their survival for hundreds, thousands and possibly even millions of years. Survival in a dry state is not restricted to such specialized bacteria: lyophilization (freeze-drying) is routinely used for long-term preservation of bacterial cultures, and, upon rehydration, the cells resume growth and divide normally. On the other hand, the presence of liquid water is essential for active growth, and microbial development ceases when water availability is severely restricted.

There are several ways to quantify water stress. To the microbiologist who deals with osmotic water stress (in which water availability is restricted due to the presence of high concentrations of salts, sugars or other solutes) the most useful parameter is water activity. It is defined as:

$$ a_w = \frac{P}{P_o} \quad \text{(in dilute solutions equal to} \quad \frac{n_2}{n_1} n_2) $$

where $P$ is the vapour pressure of the solution, $P_o$ is the vapour pressure of pure water, $n_1$ is the number of moles of solute, and $n_2$ is the number of moles of solvent. $a_w$ is related to the relative humidity ($RH$) according to:

$$ RH = \frac{a_w}{100} $$

Another parameter, used especially to quantify drought stress ('matric water stress') is the water potential, measured in bars:

$$ \psi = \frac{1000 \cdot RT}{W_A \cdot \ln(a_w)} $$

where $R$ is the gas constant, $T$ is the absolute temperature and $W_A$ is the molecular mass of water. Pure water has a water potential of zero, and all solutions have negative potentials with respect to pure water.

Figure 1 presents information on $a_w$ values of different osmotically stressed environments. Seawater containing 35 g/l salts has an $a_w$ of 0.98, and for a saturated NaCl solution, $a_w$ is 0.92.

**Key words:** bacteriorhodopsin, biological membrane, salinity, water activity, water stress

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Figure 1. Water activities of selected saline and hypersaline environments, and the lowest water activity values enabling growth of selected halophilic and osmophilic micro-organisms.
solution, $a_w=0.75$. Some salt lakes have even lower $a_w$ values. The Dead Sea, containing about 1.9 M MgCl$_2$, 1.6 M NaCl, 0.4 M CaCl$_2$, and 0.2 M KCl, has an $a_w$ value of 0.67, and a value of 0.45 was determined for the saturated CaCl$_2$ brines of Don Juan Pond in Antarctica.

A water activity of 0.60–0.62 is generally considered to be the lower limit enabling cellular metabolism and growth. The organism that tolerates the lowest $a_w$ for growth appears to be the ascomycete fungus *Xeromyces bisporus*, which thrives in concentrated sugar solutions. In hypersaline environments, the lowest $a_w$ value supporting life appears to be somewhat higher than that for the osmophilic fungi and yeasts. Currently, the Dead Sea appears to be just too extreme to support extensive growth of halophilic micro-organisms, but the presence of saturating NaCl concentrations does not preclude microbial growth. Some of these organisms cannot grow at low $a_w$ values and have adapted their entire physiology to the presence of molar concentrations of salt. The understanding of their properties can provide us with an insight into the problems related to low water availability. As discussed below, different organisms have solved the problem in different ways.

**Hypersaline environments and their microbial communities**

NaCl-saturated environments such as the crystallizer ponds of solar salterns (Figure 2) and the north arm of Great Salt Lake, Utah, are coloured brightly red owing to the presence of dense communities of halophilic micro-organisms. Predatory animals are absent from these hypersaline brines, and protozoa, if present at all, are very rare. As a result, microbial communities are generally very dense: numbers of prokaryotes between $10^7$–$10^8$ per millilitre and sometimes even higher are common, and the unicellular alga *Dunaliella salina* (see below) is often found at $10^3$–$10^4$ and more per millilitre of brine.

The community of micro-organisms found in such environments with $a_w$ values of 0.75 and even below is quite diverse and includes representatives of all three domains of life: Archaea, Bacteria and Eukarya. In saltern crystallizer ponds worldwide, the dominant type of archaeon appears to consist of flat, square to rectangular cells containing gas vesicles (Figure 3, top panel). This type of prokaryote was first isolated in 2004, and it was described as *Haloquadratum walsbyi*. It is closely related to *Halobacterium salinarum*, another member of the family Halobacteriaceae, whose properties have been studied for nearly a century. The red–purple colour of such organisms is mainly caused by carotenoid pigments (the 50-carbon carotenoid α-bacterioruberin and derivatives), and the presence of bacteriorhodopsin and other retinal pigments (see below) may also contribute to their colouration. There are also red-pigmented, extremely halophilic members within the Bacteria domain: *Salinibacter ruber* (Bacteroidetes) is no less salt-tolerant and no less salt-requiring than *Halobacterium*, *Haloquadratum* and their relatives.

We also find a eukaryotic micro-organism in salt-saturated brines: the unicellular green alga *D. salina* (Figure 3, bottom panel). In the salterns, its cells are generally coloured red–orange owing to their high content of β-carotene, present as small globules within the chloroplast. Green-coloured relatives develop in the Dead Sea when the salinity of the upper water layers is temporarily lowered because of winter rains.

**Molecular mechanisms of salt adaptation in halophilic micro-organisms**

Biological membranes are permeable to water. Therefore any micro-organism that lives in a low water activity environment, whether caused by salts or by high concentrations of sugars, will have to balance its cytoplasm osmotically with its medium. Cells that maintain a turgor pressure even need to keep a somewhat hyperosmotic cytoplasm. Halophilic archaea such as *Halobacterium* and *Haloquadratum* do not have a significant turgor pressure, but as far as is known all...
Halophilic archaea of the genus *D. salina* mainly enter the cell passively, driven by the membrane potential (negative inside), but there are indications that active transport mechanisms for K⁺ may also be operative. Active uptake of Cl⁻ against the membrane potential is mediated by halorhodopsin, another retinal protein that acts as a light-driven Cl⁻ pump, as well as by a light-independent system, probably a Na⁺/Cl⁻ symporter.

Examination of the enzymes and other cellular proteins of *Halobacterium* and relatives shows a large excess of the acidic amino acids glutamate and aspartate. Aspartate and glutamate residues are known to bind more water than other amino acids, and thus the proteins are able to maintain a hydration shell in spite of the low $a_w$ of the cytoplasm. Contents of lysine and arginine are low, so that proteins typically have a large net negative charge. In the absence of salt, such proteins generally denature. The price these organisms pay for their ability to grow at high salt is therefore the inability to survive at low salt conditions.

The 'KCl-in' strategy is not widespread within the diverse world of halophilic or halo-tolerant micro-organisms. In addition to the Halobacteriaceae, we find it in the Halanaerobiales, a small group of anaerobic fermentative prokaryotes phylogenetically affiliated with the Gram-positive branch of the Bacteria, and also the above-mentioned *Salinibacter ruber* accumulates KCl and has a predominantly acidic proteome.

*Dunaliella* uses a fundamentally different strategy: salts are excluded from the cytoplasm, and instead the alga accumulates molar concentrations of photosynthetically produced glycerol (Figure 5). Glycerol is just one representative of the long list of organic compounds known to serve as osmotic solutes. No prokaryote is yet known to use glycerol for this purpose, probably because of the high permeability of their cell membranes to glycerol. The list of organic osmotic stabilizers used by the prokaryotic world includes simple sugars (sucrose, trehalose), amino acid derivatives [betaine (1-carboxy-N,N,N-trimethylmethanaminium inner salt), ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid)], and others. Often a single organism uses a cocktail of solutes. The intracellular solute concentration is regulated to match the salinity of the medium, and micro-organisms that other micro-organisms do.

The mechanisms used by halophiles to achieve osmotic balance are highly diverse, but basically they are based on either of two fundamentally different strategies. One option is to balance the outside salts with high intracellular salt concentrations, using K⁺ rather than Na⁺ as the main cation in the cytoplasm. This option requires far-going adaptations of the entire intracellular enzymatic machinery to the presence of molar concentrations of KCl. The other option is to exclude salts from the cytoplasm, and instead to accumulate small, generally uncharged or zwitterionic, organic compounds as 'compatible' solutes. Such organic solutes do not greatly affect enzymatic activities, and therefore no great modifications of the cells' enzymes are required.

Halophilic archaea of the family Halobacteriaceae are the best-known organisms that accumulate KCl to provide osmotic balance. Figure 4 shows the main components of the bioenergetic mechanisms used by the cells to exclude Na⁺ and accumulate K⁺ in their cytoplasm. The primary driving force is the proton gradient generated across the cell membrane by respiratory electron transport. In some species, it is supplemented by proton extrusion by the light-driven proton pump bacteriorhodopsin, a retinal protein in the membrane, sometimes located in special 'purple membrane' patches. A strong Na⁺/H⁺ antiporter maintains the internal Na⁺ concentration at a low level. K⁺ mainly enters the cell passively, driven by the membrane potential.

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accumulate such organic solutes often display a large flexibility in their growth requirements, being able to adapt to life at a wide range of salt concentrations. However, energetically the price that micro-organisms have to pay for the production of these osmotic solutes while excluding salts from their cytoplasm is higher than the cost of a simple exchange of Na\(^+\) by K\(^+\) as in *Halobacterium*.

**The ultimate challenge to salt-adapted micro-organisms: life in high MgCl\(_2\) and CaCl\(_2\) concentrations**

As shown above, quite a number of micro-organisms are able to thrive in saturated NaCl solutions (\(a_w=0.75\)). However, Na\(^+\) and Cl\(^-\) are not the only ions present in hypersaline environments. Seawater also contains Mg\(^2+\), Ca\(^2+\), SO\(_4\)\(^2-\) and other ions, and some of their salts are more soluble than NaCl. Their solutions can reach \(a_w\) values far below those found in the salt marsh crystallizer ponds.

Although in the past there has been a report of the occurrence of microbial life in the CaCl\(_2\)-saturated brines of Don Juan Pond, Antarctica, later studies have not confirmed this observation. An \(a_w\) of 0.55, below which DNA becomes disordered (Figure 1), may well be the theoretical limit of water-based life. It should also be remembered that bivalent cations such as Mg\(^2+\) not only lower the water activity, but also have a destabilizing ‘chaotropic’ effect on proteins and other cellular components. Therefore the concentrations at which such ions are present, as well as the concentration ratio of such destabilizing ions and stabilizing (‘kosmotropic’) ions such as Na\(^+\), determines whether or not microbial life may be possible. In the Na\(^+\)-saturated Dead Sea, large amounts of NaCl have precipitated to the bottom in the last 25 years, leading to an ever-increasing ratio of Mg\(^2+\)/Na\(^+\). Currently, a small community of archaea and other micro-organisms still survives in the lake at an \(a_w\) value of about 0.67, but conditions have become too extreme for active growth. Blooms of *Dunaliella* and red halophilic archaea now only occur on those rare occasions when the upper water layers of the lake become sufficiently diluted by winter rain floods. Recent studies of the microbial distribution and activity along the salinity gradient between seawater and a nearly saturated MgCl\(_2\) solution in the Discovery Basin, a brine lake located 3.5 km below the surface of the Mediterranean Sea, have proved that the chaotropic effect of Mg\(^2+\) defines the limits of life here.

These and other examples show how life has adapted in different ways to high salinity. Nature has provided us with greatly disparate low-water environments, and the study of their biota can be expected to increase our insights into the ways the microbial world has learned to cope with a limited availability of the molecule most essential to all life on Earth: water.

**Figure 5.** Accumulation of glycerol as compatible solute by the halophilic/halotolerant green alga *Dunaliella*. Glycerol is synthesized within the chloroplast from photosynthetically produced dihydroxyacetone phosphate, an intermediate of the Calvin cycle. Intracellular Na\(^+\) concentrations are kept at a low level by a primary Na\(^+\) pump associated with electron transport within the plasma membrane.

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