

## Survival strategies for microorganisms in hypersaline environments and their relevance to life on early Mars

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**Abstract**—There are two groups of microorganisms that live and grow in hypersaline (>10–15% NaCl) environments: the halophilic Archaea and the halotolerant Bacteria and algae. In order to grow and reproduce in such high-salt, low-water activity environments, these organisms have made basic biochemical adaptations in their proteins, osmoregulation mechanisms, nucleic acids, and lipids. The environment of the halophiles and especially how the halophilic Archaea have adapted to that environment are reviewed in this paper. Along with this review is a brief description of how these adaptations could be important in the detection of life on early Mars assuming similar types of salts and a carbon-based life.

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### INTRODUCTION

If one thinks about salt at all, one thinks of its use on icy roads, as a flavoring in food, and possibly its use in the chemical industry. Traditionally, however, salt has been widely used to preserve hides and meats and in the preparation of sauerkraut and salted sauces. Despite the high concentration of salt used in these foods, the food or hides occasionally turned pink or had pink "salt stains" (Clayton and Gibbs, 1927). This was an especially serious problem for the salted cod industry (Kellerman, 1915; Browne, 1922) and led to the first attempts to understand the nature of this pink discoloration. It was found that the pink color was due to bacteria that grow in high-salt concentrations: the halophilic Archaea. Similarly, in the solar heating of seawater to produce salt, the increasing concentrations of salt result in changing colorations in the pans due to chemical changes. However, the green and red colorations of the various ponds are due to microorganisms: the halophilic Archaea and the halotolerant algae and Bacteria. This paper will review the characteristics of the hypersaline environment, what survival strategies microorganisms have developed in order to survive in this environment, with emphasis on the halophilic Archaea, and finally how these types of adaptations might have contributed to life on early Mars.

### WHAT IS A HALOPHILE?

As with other characteristics of microorganisms, such as growth temperature, there are gradations in the salt concentrations over which microbes are able to survive and grow. Just as some organisms will survive, but not grow, at 4 °C; there are many bacteria that will tolerate exposure to 3–8% salt but will not grow. For the purposes of this review, halophiles will be divided into two categories. The first category is the halophilic Archaea that will not grow below salt concentrations ~10–12% and may grow at up to 34% NaCl, depending on temperature, and in which the compatible solutes are K ions and Cl ions. These microbes are generally considered the obligate halophiles (Grant and Larsen, 1989). The halophilic Archaea are usually pigmented and share characteristics with both the bacterial and the eukaryotic domains.

The second category is the halotolerant Bacteria and algae, especially *Dunaliella* spp., which are algae that will grow over a broad salt range, often from essentially no NaCl to 25% NaCl, or occasionally 30% NaCl, and in which the compatible solute is neither K nor Cl ions but organic compound(s). This review will concentrate

on the survival strategies of the halophilic Archaea with some comparisons with the halotolerant Bacteria and algae.

### WHAT IS A HYPERSALINE ENVIRONMENT?

To understand the need for survival strategies in hypersaline environments, it is necessary to understand that environment. Solar salt production is based on a series of large concentration pans through which seawater is moved. At each concentrator, the salt content is increased due to the evaporation of water until finally the NaCl precipitates in the crystallizers leaving behind the more soluble K, Mg, and Mn salts in the mother liquor. Earlier in the concentration process, the Ca salts precipitated out as gypsum.

Therefore, as the salt content of a solution increases, the water content decreases per unit volume. This means that although the incoming sea water may have been ~3.3% total salt weight/volume (w/v), the final salt concentrations will be over 34% (w/v), thus, reducing the amount of available water per unit volume for the microorganisms. Hypersaline environments then are either salt crystals or those waters that contain >10% NaCl and may have a higher total salt content depending on the source. Such an environment is characterized by a low-water content or water activity ( $a_w$ ) level because of the high-salt concentration.

Water activity ( $a_w$ ) has been defined by Brock *et al.* (1994) as the "ratio of the vapor pressure of the air in equilibrium with a substance or solution to the vapor pressure at the same temperature of pure water." Values for  $a_w$  range from 0 to 1 and are unitless. Pure water has an  $a_w$  of 1.00, whereas seawater has an  $a_w$  of 0.980. Maple syrup has an  $a_w$  of 0.900, salted fish has an  $a_w$  of 0.750, and dry cereals have  $a_w$  of 0.700. Typically, growth does not occur at  $a_w$  below 0.72 (Brock *et al.*, 1994) with the halophilic Archaea approaching this lower limit. What this means to organisms is that as the  $a_w$  decreases, there is less water for dissolving nutrients, for general metabolic processes, and for hydrating proteins and nucleic acids. Therefore, organisms living in this environment must acquire compensatory mechanisms or adapt their biochemistry to cope with the low water availability.

The hypersaline environment also has other distinctive characteristics: high total ionic composition; generally stable temperatures; and, in solar salt pans, it may have intense light, alkaline pH, or the presence of alternate electron acceptors other than O<sub>2</sub>. The importance of these environmental characteristics to the survival strategies of the halophiles is discussed below.

### Ionic Composition

Besides the low  $a_w$ , the ionic composition is the defining aspect of the hypersaline environment. The exact ionic composition of brines will vary depending on whether the salt solution is derived from seawater (thalassohaline) or from other sources (athalassohaline). Specific ionic compositions of selected hypersaline lakes, both neutral pH and alkaline lakes, are listed in Table 1, along with seawater for comparison. Most of the hypersaline waters in Table 1 contain eight to ten times more total dissolved salts than seawater. However, because  $\text{Ca}^{++}$  begins to precipitate at total salt concentrations of 6–8%, most halophilic microorganisms do not require elevated concentrations of this cation for growth. Similarly, because the brines contain elevated levels of  $\text{K}^+$  and  $\text{Mg}^{++}$  (especially in thalassohaline systems), many of the halophiles will require increased concentrations of these ions for growth in artificial media.

In athalassohaline environments, other ions besides  $\text{Na}^+$  predominate. In the Dead Sea, for example, the major cations are  $\text{Mg}^{++}$  followed by  $\text{Na}^+$  and  $\text{Ca}^{++}$  (Larsen, 1980), Table 1. Therefore, the Dead Sea halophilic Archaea have their optimal growth at inorganic ion concentrations ranging from 0.6–1.2 M  $\text{Mg}^{++}$  and 2.5–4 M NaCl (Grant and Larsen, 1989) reflecting their native habitat. What is particularly interesting about the Dead Sea is that the inorganic composition did not change following the 1978–79 overturn of the lake (Javor, 1989); but the nutrient composition did change due to the massive flooding. This nutrient increase resulted in algal and bacterial blooms in 1980 (Oren, 1988). By 1991, however, the continued evaporation of the Dead Sea water had resulted in an increase in the  $\text{Mg}^{++} + \text{Ca}^{++}/\text{Na}^+ + \text{K}^+$  ratio of from 1.154 in 1977 to 1.276 (Oren, pers. comm.). Thus, successful cultivation of bacteria and algae from the Dead Sea requires a change in both the ionic composition and ionic ratio of the medium to approximate more closely that habitat.

Soda lakes also present another variation on the ionic composition of hypersaline environments. In these lakes, carbonate and chloride are the predominant anions,  $\text{Na}^+$  the dominant cation along with very low concentrations of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ . To culture microorganisms from soda lakes, therefore, adjustments in the ionic composition of the medium again are necessary to reflect the source from which the organisms originated. Frequently, in either case, the easiest way to mimic the local ion ratios and pH is to use site water.

### Compatible Solutes

To compensate for the high osmotic pressure mentioned above, compatible solutes are produced by microorganisms. These solutes may be either inorganic ions or organic molecules, and their function is to balance the internal osmotic pressure with the external osmotic pressure to maintain cellular integrity.

Sodium ions, along with many inorganic ions, will passively diffuse across the membrane. This creates a problem for microbes lacking an adaptive mechanism for dealing with the increased salt concentrations. Nonhalophilic cells when exposed to >10–12% salts will lyse because of the excessive osmotic pressure generated by the high external  $\text{Na}^+$  concentrations compared to the internal  $\text{Na}^+$  concentrations. The halophilic Archaea, on the other hand, have developed an active  $\text{Na}^+$  pump by which a  $\text{Na}^+$  gradient is established across the cell membrane so that  $\text{K}^+$  is pumped into the cell in response to the electrochemical gradient established and the  $\text{Na}^+$  is extruded. The  $\text{H}^+$  gradient can be used for either the excretion of  $\text{Na}^+$  or adenosine triphosphate (ATP) formation (Lanyi, 1993). Adenosine triphosphate is the primary energy storage compound in all living cells, so its synthesis is critically important. The maintenance of the proper intracellular ionic composition provides a mechanism for this synthesis. The overall effect is the creation of an internal  $\text{K}^+$  concentration of ~5 M that acts to balance the osmotic pressure of the ~4 M  $\text{Na}^+$  and other ion concentrations outside the cell. The movement of ions across the membrane has been nicely illustrated by Gilmour (1990), Fig. 1. This figure illustrates how  $\text{Na}^+$  is pumped from the cells, whereas  $\text{K}^+$  is concentrated within the cell.

Although the halophilic Archaea survive in the hypersaline environments through the control of inorganic ion concentrations, the halotolerant Bacteria and algae have developed an alternative osmotic pressure balancing mechanism: the synthesis of specific organic molecules as their compatible solutes. The concentrations of intracellular compatible solutes adjust to the external osmotic pressure, and each organism has a characteristic group of compatible solutes.

The halotolerant Bacteria, for example, have developed several routes for the maintenance of osmotic pressure. One adaptation is to take up the compatible solute from the environment. For example, *Halomonas elongata* concentrates glycine betaine from the environment, but it can also synthesize ectoine. Other microorganisms such as the photosynthetic halophilic bacteria can only synthesize

TABLE 1. Ionic composition of selected hypersaline bodies (g/L).

Ionic composition	Lake Magadi, Kenya	Wadi Natrun Zugm, Egypt	North Arm Great Salt Lake, USA	South Arm Great Salt Lake, USA	Dead Sea, Israel (1977 March)	Seawater	Crystallizer (at saturation), Western Salt Co, San Diego, CA
$\text{Na}^+$	46	142	105.4	35.9	40.1	10.6	120
$\text{K}^+$	0.06	2.3	6.7	3.0	7.65	0.38	52
$\text{Ca}^{++}$	0.0007	0	0.3	0.2	17.2	0.40	3.01
$\text{Mg}^{++}$	BDL*	0	11.1	4.2	44.0	1.27	14.4
$\text{Cl}^-$	14	154.6	181	65.3	224.9	18.9	210
$\text{CO}_3^-$	34.9	67.2	0.27	—	0.077	0.14	24.5
$\text{SO}_4^-$	—	22.6	27	8.7	0.45	2.65	—
Total N	0.038	—	4.3–7.2	—	—	—	—
pH	10.35	11.0	7.7	8.2	5.9–6.3	8.1	—
References	(1)	(2)	(3)	(4)	(5)	(6)	(7)

\*below detection limit.

— = not determined/not reported.

References: (1) = Grant and Tindall (1986); (2) = Larsen (1980); (3) = Post (1977); (4) = Post (1977); (5) = Beyth (1980); (6) = Brock *et al.* (1994); (7) = Javor (1983).

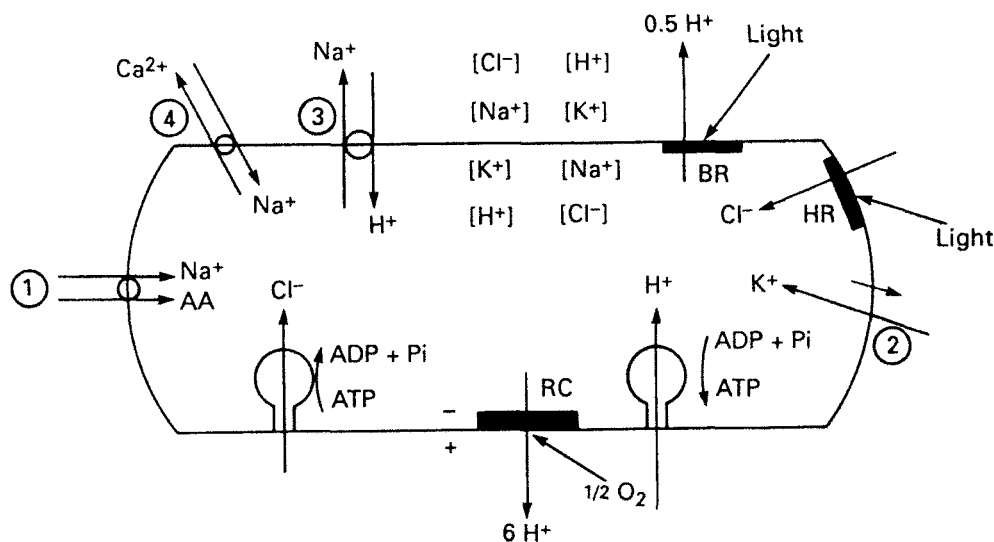


FIG. 1. The active transport of ions and amino acids into a typical halophilic Archaea. RC = respiratory chain; HR = halorhodopsin; BR = bacteriorhodopsin. (1) Active uptake of amino acids during  $\text{Na}^+$  entry (symport); (2)  $\text{K}^+$  entry in response to the electrochemical gradient set up by step (3); (3) active  $\text{Na}^+$  excretion coupled to  $\text{H}^+$  entry (antiport); and (4)  $\text{Ca}^{2+}$  excretion coupled to  $\text{Na}^+$  entry (antiport). Reproduced with permission of McGraw Hill (Gilmour, 1990).

their compatible solutes, ectoine (Peters *et al.*, 1990), or glycine betaine (Ollivier *et al.*, 1994). A third mechanism is *de novo* synthesis of glycine betaine (Louis and Galinski, 1997) or amino acids or sugars such as sucrose or trehalose (Ollivier *et al.*, 1994). Finally, the halotolerant alga, *Dunaliella*, synthesizes glycerol as its compatible solute (Ben-Amotz and Avron, 1973). Thus, the halophilic Bacteria and *Dunaliella* spp. have multiple mechanisms for osmoregulation, whereas the halophilic Archaea rely totally on inorganic salts.

### Light

One characteristic of successful solar salt works is an abundance of sunlight. This increases the evaporation rate of the water through solar heating. Consequently, the halophilic Archaea that live in these shallow evaporation pans have developed a rich array of carotenoid pigments. It is thought that these pigments provide a protective barrier to the ultraviolet light from the Sun because carotenoids contain conjugated double bonds that can function as free radical scavengers. As an added benefit, pigmented surfaces absorb more heat from the Sun; hence, heavily pigmented pans may have a temperature of 5–8 °C higher than nonpigmented pans (Litchfield, unpubl. data).

In addition, many of the halophilic Archaea contain the pigments, bacteriorhodopsin and halorhodopsin. Halorhodopsin is important in the excretion of  $\text{H}^+$  ions and the import of chloride ions into the cells, whereas bacteriorhodopsin is involved in the export of  $\text{H}^+$  ions during the process of ATP generation (Fig. 1). Unlike plants or photosynthetic microorganisms, this energy production does not involve chlorophylls but is the result of a light-dependent proton gradient established across the membrane. Thus, bacteriorhodopsin, found in the genus *Halobacterium*, allows for energy production under reduced  $\text{O}_2$  or anaerobic conditions and high light intensities. This provides a competitive advantage to *Halobacterium* spp. over those organisms that must rely totally on oxidative metabolism for energy production.

Besides the halophilic Archaea, *Dunaliella* spp. are the most common photosynthetic algae found in high-salt environments. These algae also contain high concentrations of carotenoids with  $\beta$ -carotene

predominating, which is harvested commercially. Thus, the color of ponds and salterns containing blooms of *Dunaliella* are typically a deeper red/orange than those in which the halophilic Archaea are blooming.

Most of the photosynthetic halophilic Bacteria are found in floating mats, under gypsum crusts, or along the edges of the salt pans where the light intensities are very bright (edges of the salt pans) to low (beneath gypsum crusts). They have not been found in mined salt. Both cyanobacteria and the purple sulfur bacteria inhabit hypersaline environments such as solar salt pans (Litchfield, 1977; Javor, 1989) and the shores of inland salt lakes such as Hamei Mazor along the Dead Sea (Oren, 1989). These microbes are anoxygenic photoautotrophs, which do not produce  $\text{O}_2$  from photosynthesis but instead fix C via bacterial chlorophylls. Thus, these hypersaline Bacteria are adapted to up to ~25% salts as well as variable light intensities. Many of these microbes are also S oxidizing. Therefore, they constitute a metabolically versatile group of microorganisms that have not been widely investigated but were reviewed recently by Ollivier *et al.* (1994).

While the photosynthetic Bacteria are generally pigmented, the halotolerant Bacteria may or may not be pigmented. One of the most widely distributed organisms in a solar salt plant is the genus *Halomonas*. To date, none of the isolates from this genus have been shown to be pigmented despite their growth or survival in salt concentrations similar to those of the pigmented halophilic Archaea.

### Temperature

Because most solar salt production plants are in tropical regions, there is relatively little temperature fluctuation for the halophiles. Most of the pure culture isolates examined to date tolerate at least 15–20 °C, even if they do not grow at these lower temperatures. The optimum temperature tends to be in the 35–55 °C range (Grant and Larsen, 1989), which, again, is reflective of their environment.

### pH

The various pans in solar salt plants generally have a pH in the neutral to slightly alkaline range of 7.1 to 8.1. As the water becomes more saturated, the pH decreases somewhat so that crystal-

lizer pH values tend to be in the 6.2–7.2 range (Litchfield, 1977). The pH optima of the halophilic microbes is usually between 5.5–8.5 (Grant and Larsen, 1989). For those microorganisms growing in soda lakes, however, the pH range is typically 8–10 (Grant and Larsen, 1989). Thus, the halophilic Archaea and halotolerant Bacteria live within a fairly restricted pH range typical of their native environment.

### Electron Acceptors

For solar salt pans that typically have a significant amount of wind, the constant agitation of the surface ensures that O<sub>2</sub> is available to the microorganisms in the upper layers of the water. Thus, members of the Halobacteriales, the halophilic Archaea, are all aerobic or facultatively anaerobic, which means that they either use O<sub>2</sub> for aerobic metabolism or nitrate as their terminal electron acceptor. Nitrate is frequently present in solar salterns but at fairly low levels, 0–4.2 mg/L (Litchfield *et al.*, 1998). Thus, it is uncertain to what extent nitrate can serve as an alternate electron acceptor for the halophiles in their environment. To date, no obligately anaerobic halophilic Archaea have been isolated, but it seems likely that they exist because of the amount of sulfate present in the sediments and the anaerobic nature of the bottom sediments in the high salt pans.

However, truly anaerobic halotolerant Bacteria have been isolated from hypersaline lakes and solar salt plants (for example, Oren, 1983; Rengpipat *et al.*, 1988, for fermentative strains; Zhilina, 1986; Yu and Kawamura, 1987, for methanogens; see Ollivier *et al.*, 1994, for a review of this area). Because of the relatively few studies on anaerobic halophilic Bacteria, it is not known to what extent these organisms contribute to the nutrient cycling in salt pans. Sulfate reduction has been observed both experimentally and in the field, so it is likely that organisms such as *Desulfohalobium* sp. (Ollivier *et al.*, 1991) are important members of the microbial community. In fact, the recent isolation of an alkaliphilic halotolerant sulfate-reducing eubacterium from Lake Magadi lends support to the hypothesis that these organisms are responsible for the lack of sulfate in soda lakes and are the cause of the high alkalinity of these bodies of water (Zhilina *et al.*, 1997).

In summary then, the halophilic Archaea are generally aerobic, whereas the halotolerant Bacteria may range from obligate aerobes to photoautotrophs to anaerobic sulfate-reducers bacteria. So, despite the high-salt concentrations, metabolic diversity is common to solar salt works, even in the crystallizers (Litchfield *et al.*, 1998).

### SURVIVAL STRATEGIES

Given the apparently harsh environment of hypersaline lakes and salterns, it may seem surprising that a diverse and interesting group, including both prokaryotic and eukaryotic microorganisms, could form complex functioning communities in these waters. Survival strategies are needed at all salt concentrations, but especially at salt levels >15% (w/v). This is especially true for the halophilic Archaea with their high internal K<sup>+</sup> concentrations. The survival strategies for these microorganisms have required biochemical adaptations to their proteins, lipids, and nucleic acids.

#### Proteins

Halophilic archaeal proteins have been modified to contain high concentrations of acidic amino acids (glutamic acid and aspartic acid). The excess acidic amino acid residues range from 12–20 mol%; for comparison, *Escherichia coli* has from 0.2 to 9 mol% excess acidic amino acids (Lanyi, 1974). In the halophiles, most of these acidic amino acids are found on the external surface of the protein (Eisen-

berg *et al.*, 1992) and may be found around the N-termini that helps to stabilize the helices through salt bridges (Dym *et al.*, 1995). Because the internal concentration of K ions is almost equal to the external Na<sup>+</sup> concentration, the ionic interaction between the negative charges on the proteins and the K ions (which hydrate less water than Na<sup>+</sup>) helps to form a hydration shell around the proteins allowing them to continue to function (Dennis and Shimmin, 1997). This hydration shell also reduces the electrostatic free energy of the proteins (Lanyi, 1974).

In addition, many enzymes require elevated K<sup>+</sup> to maintain their activity. This requirement may be either ion specific or Na<sup>+</sup> or other cations may substitute depending on the enzyme, so no generalizations can be made. Hydrophobic bonds may also be important in the maintenance of protein stability and activity. It has been postulated that one effect of the cation requirement is to help overcome unfavorable steric conditions affecting the packing of the nonpolar groups on valine, isoleucine, and leucine. If there is inefficient interior packing of the protein, the interior may be too loose to permit enzymatic activity due to altered configurations in the active site (Lanyi, 1974). Hence, the active centers may be protected by the interior location of the hydrophobic groups.

The proteins of Bacteria growing in hypersaline environments also tend to have higher concentrations of the acidic amino acids than similar types of proteins from nonsalt-tolerant eubacteria. Among the first enzymes from halophilic Bacteria to be isolated and characterized, the protease from a moderately halophilic *Pseudomonas* sp. was found to have 16 mol% of excess acidic amino acids over basic amino acids (Qua *et al.*, 1981). This is within the range of the halophilic Archaea and suggests that the compatible solutes may play a role in protein stabilization similar to that of the cations in the Archaea. How these organic compatible solutes interact with the internal structures of the hypersaline Bacteria has not been thoroughly investigated.

Another adaptation involves the cell wall. The external cell walls of the halophilic Archaea contain a glycoprotein instead of the outer membrane/peptidoglycan structure of the Bacteria. Again, there is in the glycoprotein an excess of acidic or negatively charged amino acids, and the cell walls may also contain sulfate, another negatively charged molecule. As with the internal proteins, these negative charges allow for a hydration shell around the cells possibly increasing the solubility of needed nutrients and proper functioning of transport mechanisms. This aspect of halophilic Archaeal structure has been reviewed by Wieland (1988).

In addition, in the archaeal ribosomal 5S proteins, which are more similar to those found in eukaryotic organisms, the acidic amino acids again predominate: glutamic acid is the most common followed by equal concentrations of aspartic acids, valine, alanine, and glycine (Kimura *et al.*, 1989).

From this very brief overview, it is obvious that the ionic environment and the acidic nature of the proteins involve a complex series of interactions. But the overall effect is to stabilize the protein, provide an aqueous environment, and insure its proper functioning whether the protein is found on the exterior cell surfaces or internal in the cytoplasm.

#### Lipids

All Archaea, including the halophiles, contain a unique set of lipids. The lipids do not contain the typical fatty acids found on the two and three carbons of the glycerol backbone in the Bacteria and Eukarya. Instead, archaeal lipids are composed of ether linkages to the glycerol. In addition in the halophilic Archaea, these ether-

linked side chains are comprised of four to five branched isoprenoid units (Fig. 2). This combination is more stable in the high-ionic environment while maintaining the proper membrane fluidity to allow for the passage of molecules across the cell wall/membrane complex.

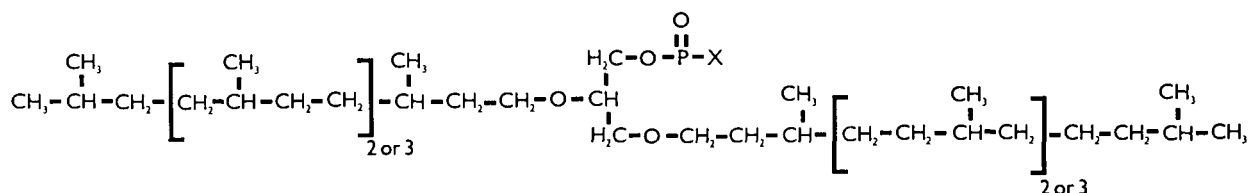
Phospholipids are those glycerol lipids to which a phosphate is linked on the first carbon (Fig. 2), and they are found in all living systems (Kates and Moldoveanu, 1991; Matsubara *et al.*, 1994). However in the Archaea, this phosphate may be substituted with glycerol or phosphate moieties resulting in a distinctive class of phospholipids. Additionally, some of the polar lipids may not contain phosphate but have direct substitution of glucose onto the first carbon in the glycerol backbone resulting in a class of lipids called glycolipids. These glycolipids may also contain additional sugars and/or sulfate (Kates and Moldoveanu, 1991; Matsubara *et al.*, 1994). Typical structures of these various classes of polar lipids are shown in Fig. 2. Thus, the presence of diether polar lipids is indicative of the presence of the Domain Archaea and not just the halophilic Archaea. Because many of the Archaea live in extreme environments, these unusual polar lipids appear to be essential to maintenance of the outer and cytoplasmic membranes by being less prone to hydrolysis. This stability is indicated by the fact that, to date, no archaeal ether-lipid lipases have been found in the obligate halophiles; this indicates that these molecules do not have a rapid turnover rate.

#### Nucleic Acids

Although many bacteria have high guanine (G) to cytosine (C) ratios in their DNA, all known halophilic Archaea typically contain from 59.5 to 71.2% G + C (Grant and Larsen, 1989) with most strains having at least one minor component in a slightly lower and narrower range of 55–58% G + C (Joshi *et al.*, 1963; Pfeifer, 1988). The high G + C content allows for extra H-bonding that is thought to increase the stability of the DNA when exposed to the high intracellular cation concentrations. Whether other compatible solutes such as glycine betaine also form additional H-bonds with DNA has not been reported, but it is interesting to speculate that this is a possibility when one considers that the G + C content of the extremely halotolerant genus *Halomonas* is also in the 60% G + C range (Vreeland *et al.* 1980).

### Phospholipids

X = Glycerol  
= Glycerol Phosphate  
= Glycerol Sulfate



### Glycolipids

R = Phytanyl Chains diagrammed above  
Sugar<sub>1</sub> = Glucose  
Sugar<sub>2</sub> = Mannose with Substitutions of Sulfate,  
Galactose, Glucose, etc. on C-2 or C-6.

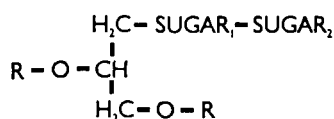


FIG. 2. Schematic diagram of some representative polar lipids (archeol lipids) of the halophilic Archaea.

The major chromosome of the halophilic Archaea contains 1.8–3 Mb and is fairly conserved in each strain. This chromosome is smaller than that of *E. coli* and contains sections homologous to both eukaryotic and bacterial chromosomes implying a more primitive and more ancient DNA (Woese, 1987). The minor component is covalently closed circular DNA and contains 80–160 kb (Pfeifer, 1988).

Besides the main chromosome and the minor component(s) mentioned above, extrachromosomal elements are common. Typically, there are two to four of these with a size of 120–400 kb or occasionally smaller. These elements can account for up to 30% of the genomic material. Furthermore, these elements are highly variable, even within the same species, especially if those species are obtained from different sources. In a few cases, these have been shown to be phages with relatively high G + C ratios (62–70 mol%) and 29 to 59 kb in length (Pfeifer, 1988). Other explanations for this extra DNA is that they are plasmids, transposons, or simply repeat sequences (Meyers, 1995). Regardless, the small size of the major DNA component should not be considered as the only genetically important material in the cell. Whether these extrachromosomal elements are a result of the high internal salt concentration causing a dissociation of the DNA into smaller fragments to maintain stability is not entirely clear, but this is a common characteristic of the halophilic Archaea.

#### APPLICABILITY TO LIFE ON EARLY MARS

There appear to be at least two theories about the atmosphere of early Mars that have been summarized by Forsythe and Zimbleman (1995). One is that the atmosphere was not very different from today and the other is that there have been drastic changes in the atmosphere with a complex hydrologic cycle. Assuming that the atmosphere has evolved in a manner reviewed by Zent (1996), the climate on Mars may once have been more compatible to life as we know it. Carr (1986) states that the atmosphere may have been not only warmer but thicker than today with significant amounts of CO<sub>2</sub>. Coupled with this CO<sub>2</sub>, other gases such as methane and ammonia could also have been present (Clifford, 1993). These gases could have provided the C and N sources for photosynthetic microbial life.

There are two additional aspects of what we understand about Mars that are pertinent to suggesting that organisms similar to halophilic Archaea could have been early life forms on Mars. The first is the presence of water (Carr, 1979, 1986), thus, potentially providing a mechanism for brine solutions and eventually the second aspect: evaporite formation containing NaCl (Malin, 1974; Zent and Fanale, 1986; Forsythe and Zimbelman, 1995). This combination implies that salt solutions existed at one time; and presumably as the water froze, the salt solutions became more concentrated, thus, providing an environment for the growth of halophiles.

The Viking XRFS experiment showed the Martian surface to have the following mineral abundances: SiO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, MgO, Al<sub>2</sub>O<sub>3</sub>, and CaO with sulfate and chloride also present (Zent and Fanale, 1986; Banin *et al.*, 1992; Blaney and McCord, 1995). Working with pure solutions, Brass (1980) had earlier shown that MgCl<sub>2</sub> or CaCl<sub>2</sub> depresses the freezing point of salt solutions below -23 °C and that the addition of sulfates decreases the freezing temperature to -63 °C. However, at temperatures > -60 °C (higher than 210 K), evaporites could form. It is possible that some of these evaporites contain fluid inclusions.

Clark and Van Hart (1981) reported that the composition of brine from Eddy County, New Mexico approximated that to be expected from a similar volumetric extraction of Martian soil. Both Mg<sup>++</sup> and SO<sub>4</sub><sup>--</sup> ions dominated at a salinity about one-half that of the Dead Sea or ~17% salts, and this salinity is certainly within the range of the obligate halophiles. They also concluded that stable brines could not presently exist on Mars (Clark and Van Hart, 1981), but Zent and Fanale (1986) concluded that unstable brines could exist and these could be a chloride-sulfate system. Therefore, either historically or currently, brines could exist on Mars, thus, providing a high-salt aqueous medium for the survival and growth of organisms with characteristics similar to the halophilic Archaea.

That microorganisms can survive in salt crystals and salt inclusions has long been recognized. For over 35 years, bacteria have been isolated from salt deposits (Reiser and Tasch, 1960; Tasch, 1963; Dombrowski, 1961, 1963), with more recent isolations performed by Norton and Grant (1988), Rothschild (1990), Vreeland *et al.* (1998), and Vreeland and Powers (1998). The initial isolations by Reiser and Tasch (1960) were of diplococci, whereas Dombrowski (1961) reported motile rods. Norton and Grant (1988) showed the presence of bacteria within salt crystals obtained from Puerto Rico and Lake Magadi, Kenya. They also demonstrated that, except for *Halococcus*, all strains tested could be entrapped in the water inclusions within salt crystals. From many of these inclusions, viable cells could be cultured later, but nonviable cells were also trapped inside the crystals.

Similarly, Vreeland *et al.* (1998) also reported that occasionally halophiles could be isolated from inside salt crystals but that this was not a constant occurrence. Special procedures for the recovery of bacteria from salt crystals without external contamination have been developed, and Vreeland and Powers (1998) have noted the importance of dating and the selection of primary inclusions to conclusively state that the microorganisms, indeed, did come from within that salt crystal.

These data show that failure to cultivate microorganisms from some salt crystals may reflect whether the original microbe was dead or alive at the time of entrapment in the crystal. Certainly, live halophilic bacteria can be cultured from some of the primary inclusions in salt crystals. To date, there have been no reports about the

use of molecular techniques to test for DNA or RNA in primary inclusions that have failed to yield live organisms.

Once it is accepted that viable halophilic Archaea can survive in salt crystals, the next question concerns their metabolic state. There have been few studies reporting metabolic activity *in situ* in evaporites. Recently Rothschild *et al.* (1994) demonstrated the fixation of N and C in crushed evaporites by the cyanobacterium *Synechococcus* Nægeli. However, maximum uptake occurred at zero salt concentration implying that the organisms were more halotolerant than obligate halophiles. The authors also found that N fixation occurred under both light and dark conditions and that the cells did not apparently utilize the nitrate and nitrite present in the evaporite. Based on the chains formed by the cyanobacterium, they also concluded that cell division was apparently occurring within these thin evaporites (Rothschild *et al.* 1994). Similar work needs to be done with inclusions where halophilic Archaea have been found to see if the cells are metabolically active or quiescent.

In conclusion, the ability of halophilic Archaea to survive within low-water activity environments such as evaporites and their requirement for elevated salt concentrations makes them likely candidates for life on early Mars. This ability to survive is based on the biochemical adaptations in their proteins, nucleic acids, and lipids to their native environment. The persistence of their polar lipids on Earth could provide a biomarker for detecting their current or past presence in meteorites, fossils, or other planets. Currently, our analytical methods for detecting very small quantities of the diphytanyl diether lipids prevent the examination of inclusions or the interiors of evaporites for these fossil remains. However, this is a methodological problem that eventually will be overcome.

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