

Why MECHOs are important to bacteriologists", Commentary by Carl H. Gibson.

Abstract

HGD cosmology shows that life begins at 200 million years, soon after the plasma to gas transition, when the temperature decreases to the triple point of water. PGC clumps of dark matter planets host the formation of DNA life when water becomes available on the planets. MECOs form at the center of proto-galaxies and distribute the life to the rest of the cosmos by plasma jets of the MECO.

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Halophilic Archaea and the Search for Extinct and Extant

Life on Mars

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Abstract

Halophilic Archaea have adapted to a life in the extreme but could they thrive on a different planet? And even if they cannot survive there today, would those organisms leave detectable traces of their past existence for us to find? On Earth, halophilic Archaea can be found in many different environments, yet two of those environments are of particular interest in the search for extraterrestrial life: modern stromatolites and ancient halite. Stromatolites may have been around for 3.5 billion years and could have probably been the first microenvironments sheltering life. Halite has been shown to preserve living organisms for more than 400 million years. Both, stromatolites and ancient halite have been found to be habitats for one archaeal family, the family *Halobacteriaceae*. An intriguing location to search for halophiles (or their remnants) outside Earth is Mars, as it may have been a wetter and warmer place in the past and recent data suggest to the presence of halite on Mars. This review provides a brief overview of halophilic Archaea, their environments, their biomarkers, and the possibility of finding them on other planets.

Keywords: Halophilic Archaea, halite, Mars, stromatolites

1. Introduction

Most of our universe appears to be a hostile place for life to exist with no planetary bodies except Earth harboring life as we know it. However, similar notions were previously thought of Earth's extreme environments such as acidic hot springs, deepsea vents or solar salterns, which were believed to be too extreme to nurture life. Yet numerous studies over the last decades have shown that these extreme environments actually harbor an incredible diversity of Eukarya, Bacteria and Archaea (Rothschild and Mancinelli 2001; van der Wielen et al. 2005). The very same may hold true for the search for extraterrestrial life: Just because we have not found it yet, does not mean it cannot exist. However, there is still the question of what are we actually looking for, and where?

Since Mars and Earth are Solar System neighbors, they most likely shared certain early geological processes (Beatty et al. 2005, Nisbet and Sleep 2001). Many studies draw a picture of early Mars as being a warmer and wetter planet with a substantial amount of water, at least earlier in its history (Clifford 1993; Mc Kay 1989; Squyres et al. 2004). With this assumption in mind, Mars is probably our best chance to find life, extant or extinct, within our Solar System and recent results from the Phoenix Mars Lander have actually shown evidence for water in modern day Martian soil (http://www.nasa.gov/mission_pages/phoenix/news/phoenix-20080731.html). Another intriguing find was made by the Mars Rovers *Spirit* and *Opportunity*, when they discovered halite and sulfate evaporated rocks on Mars. This suggests that hypersaline brine pools may have been relatively common on the surface of Mars (Marshall et al. 2007), which in turn may have been a suitable environment for a family of Archaea which thrive on Earth: the family *Halobacteriaceae*. On Earth, modern hypersaline brine pools are not solely inhabited by halophilic Archaea. Two examples of other inhabitants are *Salinibacter ruber* sp. (Antón et al. 2002) or the unicellular green algae *Dunaliella salina* (Bardavid et al. 2008; Oren 2005). Both can be found in great numbers in hypersaline brine pools (Oren 2005).

2. Stromatolites

In the search of extraterrestrial life, halophilic Archaea are of particular interest as they are amazingly robust organisms, able to survive being desiccated into a crust of solid salt. Sealed in such salt crystals, halophiles have an extremely high, and perhaps indefinite, longevity (Grant et al. 1998; Landis, 2001). Interestingly, these halophilic Archaea are not known to form spores, thus it is of great interest how they can survive for an extended period of time (Radax et al. 2001). Modern stromatolites are another very interesting environment where halophilic Archaea are found (Goh et al. 2006; Allen et al. 2008; Burns et al. 2009). Ancient stromatolites date as far back as 3.5 billion years and may have provided the first micro-environments on early Earth, as they were fashioned in ancient oceans, which may have been 6% NaCl (Monty 1977; Knauth 2005).



Stromatolites, Shark's bay



Proterozoic Stromatolites.

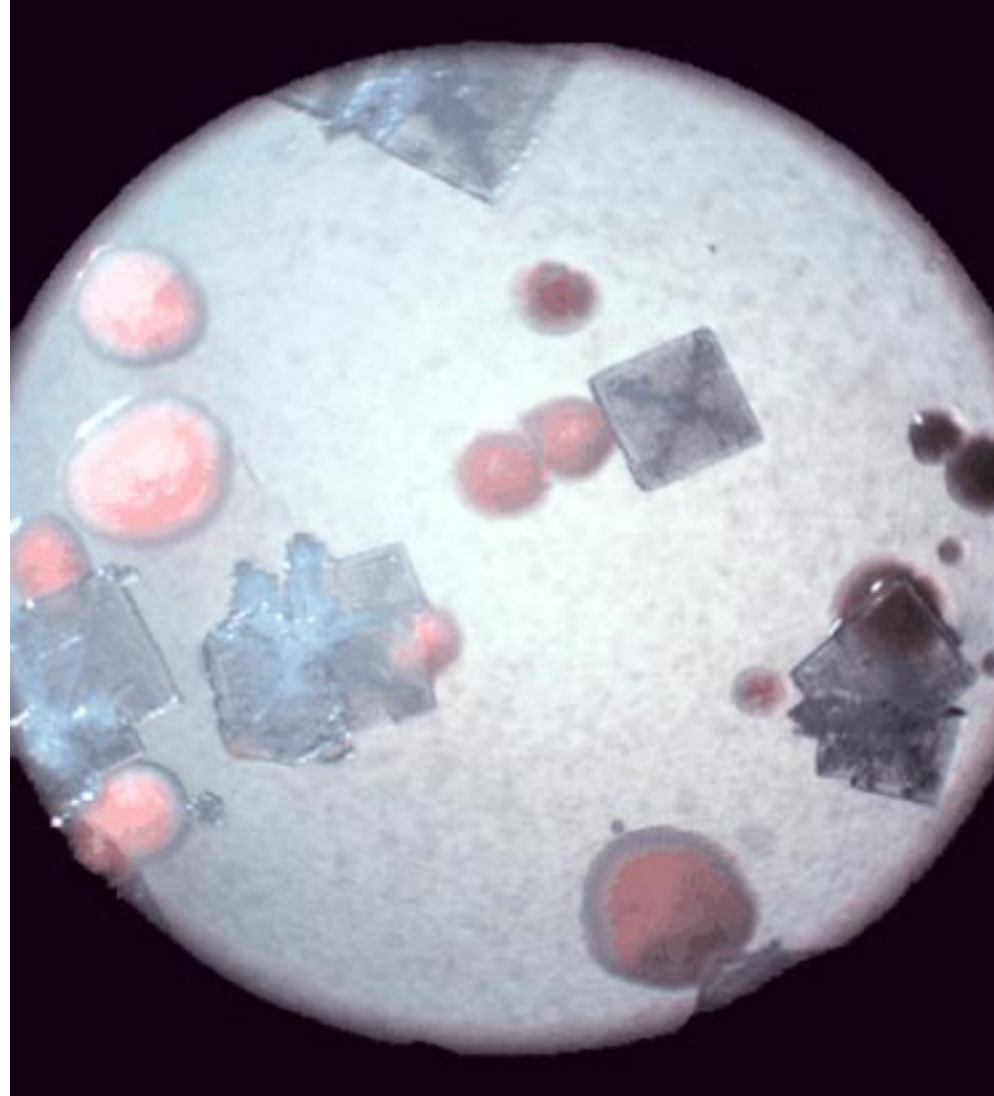
Not only is there the suggested relative common occurrence of hypersaline environments on Mars in its early history, but one can also imagine that any simple microorganisms could interact in some way with their physical environment to form similar "Earth-like" mats or stromatolites. Thus it is not unthinkable if life were to exist on early Mars that stromatolites were a common occurrence in the past, and which may have harbored halophilic Archaea. Once water on Mars started to evaporate, forcing any stromatolites to become extinct, halophilic Archaea may have become entrapped in halite where they continued to flourish.

Halophilic Archaea may survive for millions of years enclosed in salt crystals (Vreeland et al., 2000). This makes them prime candidates for organisms that may have been present on early Mars and raises the possibility that even nowadays, they may be enclosed and dormant, trapped in a crystal.

3. Characteristics of Halophilic Archaea

Halophilic Archaea have adapted to a living in extremely hostile environments. Halophilic Archaea are chemoorganotrophs and belong to the class Euryarchaeota. The principal morphological types of these haloarchaea are rods, cocci and irregular pleomorphic forms (Fendrihan et al. 2006). The so called "Walsby square archaeon" (first described in the 1980s (Walsby 1980), recently isolated and named *Haloquadratum walsbyi* (Burns et al. 2007) exhibits a very unusual cell form and are almost perfect quadratic (Walsby 2005). Halophilic Archaea (whose name comes from Greek for "salt-loving") thrive even in concentration of salt five times greater than the salt concentration of the ocean and in salt concentrations higher than those used in any food pickling processes. They in fact require salt for growth and they are adapted to environments which have little or no oxygen available for respiration. Instead, their cellular machinery contains charged amino acids on their surfaces, which react to the salt.

This Figure is from the cover art of the Journal of Bacteriology, Volume 186, issue 10, 2004. Copyright permission is pending: J of C Editors CHG and RES Aug. 2016. It should be noted that the article (Leuko et al, Journal of Cosmology 2010, vol. 5, 940-950) is now somewhat obsolete since it lacks reference to modern Astrobiology and many biological advances from HGD cosmology and Wickramasinghe/Hoyle cometary panspermia.



Halophilic Archaea

They are also pigmented in shades of red, orange, pink or purple, which captures sunlight to drive a proton pump (bacteriorhodopsin) which enables them to obtain energy for growth (Fendrihan et al. 2006). The proteins of halophilic Archaea are highly adapted and engineered to function in their natural environment, which usually contains between 2 and 5 M inorganic salts (Dennis and Shimmin 1997). Another interesting feature is that the genomic structures of these organisms have adapted to lower the occurrence of potential lesions induced by the natural occurring high UV radiation within their environment (Zhou et al. 2007). Thus they can survive in the absence of ozone.

4. Typical & Non-Typical Environments for Halophilic Archaea

Halophilic Archaea thrive in many different environments ranging from the Dead Sea (Oren 1983) to solar salterns (Benlloch et al. 2001; Litchfield and Gillevet 2002; Ochsenreiter et al. 2002). They have even been isolated from the nostrils salt glands of the Seabird *Calonectris diomedea* (Brito-Echeverría et al. 2009). All these environments are characterized by a very high sodium chloride content. However, halophilic archaea have also been found in environments low on sodium chloride such as the river Colne in Essex, UK (Purdy et al. 2004), Zodletone Spring (Elshahed et al. 2004) and modern stromatolites in Shark Bay (Allen et al. 2008; Allen et al. 2009; Burns et al. 2004; Goh et al. 2006; Leuko et al. 2007; Leuko et al. 2008; Burns et al. 2009; Goh et al. 2009). Thus they thrive in exceptionally dynamic environments with respect to light, salinity, temperature, pH and oxygen (DasSarma et al. 2001), which is a further demonstration of their adaptive versatility in response to different kinds of stress situations.

For example, *Halobacterium* NRC-1 adapt to high and low salt concentrations (Coker et al. 2007; Leuko et al. 2009), desiccation (Kottemann et al. 2005;), ionizing radiation (deVeaux et al. 2007; Kottemann et al. 2005; Whitehead et al. 2006), transition metals (Kaur et al. 2006), different temperatures (Coker et al. 2007; Shukla 2006) and different regimes of UV radiation (Baliga et al. 2004; McCready et al. 2005).

Furthermore, *Haloarcula* sp. was able to survive a 2 week exposure to the space environment while in Earth orbit aboard the Biopan facility (Mancinelli et al. 1998). Other experiments conducted by Stan-Lotter et al. (2002) showed that *Halococcus dombrowskii* and *Halobacterium* sp. NRC-1 are able to survive a simulated Martian atmosphere (6 mbar pressure, 98 % carbon dioxide, and an average temperature of -60°C), for up to 6 hours. This incredible robustness and ability to survive and adapt to different and extreme environmental stresses makes these archaea of great interest in the search for extraterrestrial life.

Halophilic Archaea have been found in two habitats, stromatolites and halite crystals, which have important implications for their ability to also thrive in extra-terrestrial environments. Ancient stromatolites may offer clues to the evolution of life on Earth, and possibly Mars, as they have been present on Earth for 3.5 billion years and may have been one of the first microenvironments to harbor early life. At this point, it needs to be acknowledged that the biological origin of ancient stromatolites is still controversial with opinions divided between diverse inorganic (Brasier et al. 2005; Brasier et al. 2006; Grotzinger and Rothman 1996; Lowe 1994) or biosedimentary origins (Allwood et al. 2006; Schopf 2006; Walter and Heys 1985). However, this review is not intended to address this debate. Nevertheless, it is reasonable to assume that at least some ancient stromatolites have been formed due to biosedimentation.

Modern stromatolites are organo-sedimentary structures constructed by the entrapment and binding of sediments by cyanobacteria and other microorganisms (Chivas et al. 1990). The microbial ecosystem on the top layer of the stromatolite plays the role of a filter that enhances, inhibits or passively allows the growth process (Dupraz et al. 2006). Thus, the formation of stromatolites results from interactions and balance between intrinsic (microbial mat and biofilm) and extrinsic factors (environmental conditions). Many important steps of evolution may have also

occurred within stromatolites owing to the close proximity of diverse microorganisms and microniches (Nisbet and Fowler, 1999), and it may have happened, or is happening now, on other planets with similar environmental conditions. If so, then halophilic archaea may be among the inhabitants.

5. Mars & halophilic Archaea

The most abundant and diverse modern stromatolites in marine environments occur in Hamelin Pool, a hypersaline embayment of Shark Bay, Western Australia (Reid et al. 2003; Burns et al. 2004; Goh et al. 2009). By analogy, ancient oceans are hypothesized by some to have had similar salinity as Shark Bay, which is around 6 % w/v (Monty 1977; Knauth 2005). As previously stated, Mars and Earth may have shared similar geological processes and Mars possibly even had an ancient ocean. If so, then stromatolites may have formed on both Mars and Earth 3.5 billion years ago (Walter and DesMarais 1993).

Halophilic archaea on Earth also thrive within subterranean salt formations, which represent the remains of ancient high salinity waters that evaporated in the distant past (Javor 1989; Park et al. 2009). Similar conditions may prevail on modern Mars.

As halite crystallizes, representatives of the haloarchaea become incorporated into a viable state within tiny pockets of brine called fluid inclusions that become a permanent feature of the crystal structure (Grant et al. 1998; Norton and Grant 1988). This scenario is well documented on Earth and recent observations from the Mars Exploration Rover suggest an ancient evaporative environment on Mars. This has raised the possibility that Martian life may thrive in these brines (Mancinelli et al. 2004; Pasteris et al. 2006; Rothschild 1990; Rothschild et al. 1994).

The first studies to describe the organisms living within halite were Dombrowski (1963) and Reiser and Tasch (1960). Since then, several extremely halophilic Archaea have been isolated from such deposits (ranging in age from 250 million years to 419 million years) e.g., *Halococcus salifodinae* (Denner et al. 1994), *Halococcus dombrowskii* (Stan-Lotter et al. 2002) and *Halobacterium noricense* (Gruber et al. 2004). The microbial content of ancient rock salt is generally low ñ estimates range from 1-2 cells/kg of salt from a British mine (Norton et al. 1993) to 1.3×10^5 colony forming units (CFUs) per kg of alpine rock salt (Stan-Lotter et al. 2000; Fendrihan et al. 2006). Although this environment is certainly not ideal for halophilic Archaea, it offers good protection against some other extremes like UV radiation.

Fendrihan et al. (2009a) showed that artificial salt crystals protect entrapped halophilic archaea against high UV radiation with 37% percent survival following a dosage of 420 kJ/m^2 for *Hcc. dombrowskii*. The potential survival of *Hcc. dombrowskii* in halite was therefore calculated to be up to a dose of at least 3000 kJ/m^2 (Fendrihan et al. 2009a). The surface of modern day Mars is exposed to a high dose of UV radiation every day. Hence, the protection halite offers to entrapped halophilic Archaea may be imperative to the long-term survival of any similar microorganisms (past or present) on Mars.

6. Detecting Biological Markers of Martian Halophilic Archaea

Microbial life, if extinct or extant on Mars, would produce biomolecules that might be preserved and detectable in Martian rocks (Marshall et al. 2006). A biomarker is a specific cell constituent produced by microorganisms and when detected, conclusively shows that living organisms are or were present in the environment. Examples of biomarkers are lipids, steroids, and pigments (Simoneit 2002). Halophilic Archaea are mostly pigmented red due to a high content of C_{50} carotenoid pigments (α - bacterioruberin and derivatives) in their membranes (Kushwaha and Kates 1979; Liaaen-Jensen 1979). In some cases this may be accompanied by the purple retinal pigment bacteriorhodopsin (Oren 2002). These pigments, in particular bacterioruberin, are known to be potent free radical scavengers and offer protection against UV radiation (Shahmohammadi et al. 1998). Recent studies have shown, that these pigments can be detected by Resonance Raman spectroscopy which is a spectroscopic technique used to study vibrational, rotational, and other low-frequency modes in a system.

Raman spectroscopy is mostly viewed as a specialist laboratory or research technique. However, in recent years several systems have been specifically developed for field based applications. Consequently, the potential use of Raman spectroscopy in planetary exploration is now being realized (Marshall et al. 2007). Previous Raman studies by Edwards and colleagues (Edwards et al. 2003, 2005a, 2005b, 2006) regarding the evolutionary ancient cyanobacterium *Nostoc* sp. (among others) have shown spectral signatures for carotenoids, hopanoids, isoprenoids and when cells were exposed to UV radiation, scytonemin.

Furthermore, a recent study by Fendrihan et al. (2009b) showed that this methodology can also be used to detect halophilic archaeal C_{50} carotenoids (mainly bacterioruberins) in laboratory-produced halite crystals. This method can be used for the analysis and remote detection of carotenoid pigments from halophilic Archaea in situ without the need for large sample sizes and extraction, which is required by analytical techniques such as high performance liquid chromatography, liquid chromatography and mass spectrometry (Marshall et al. 2007). These studies indicate that Raman and Resonance Raman spectroscopy are indeed powerful and important tools in our search for extraterrestrial life, should it be alive or dormant in a salt crystal.

7. Conclusions & Further Directions

The past decade has seen a rapid increase in technology and possibilities to look for life on different planets. Further mission to Mars e.g., the Mars Science Laboratory set for launch in 2011 or the MAVEN orbiter set for launch in 2013, will undoubtedly increase our understanding of the history of the red planet and probably offer insights into our own evolution. One of the best ways to learn more about the limits of life, stress response and adaptive mechanisms, is to study modern extreme environments. Studying modern day analogue environments will not only provide us with information about the evolution of life on our planet, but may give clues to the evolution of life on a different planet, which we then can look for on these further missions.

With all the evidence pointing at the moment to a warm and wet early Martian environment, it may be conceivable that life was thriving in a hypersaline ancient ocean on early Mars. Similar to the modern day environment of Shark Bay, stromatolites may have been present at the time, harboring and sheltering life. Once the environment was changing, in particular the loss of water, organisms may have been entrapped within forming salt crystals. Those crystals containing halophilic Archaea perhaps may still be lying dormant beneath the Martian surface, waiting for us to find them.

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The Leuko et al 2010 article does not reference the work of Hoyle/Wickramasinge that shows the origin of life everywhere in the cosmos is cometary panspermia and HGD cosmology. The comets are formed by mergers of earth mass primordial fog particles from the time of plasma to gas transition ~ 300,000 years when the planets condensed in dense clumps of a trillion, sufficient to form a million stars. The dense clumps are termed PGC, or proto-globular-star-clusters, since all stars are formed within such 10^{36} kg clumps that make up the dark matter of all galaxies. All galaxies show globular clusters that have this same mass. All galaxies fragmented to form protogalaxies with 10^{43} kg mass when the Hubble scale ct matched the Schwarzschild viscous scale of the plasma at time t of 10^{12} seconds (30,000 years), where c is the speed of light 10^8 m/s. Because 10^{80} primordial planets were formed which make all the stars by mergers within the PGC clumps, and because the chemicals of life were produced by the stars and their explosions and spread to the planets, the first life began when the water began to condense as the planets cooled to the triple point temperature at 200 million years. The planets and their oceans formed a cosmic primordial soup where the DNA RNA of all biology formed. Biology is a subset of astrobiology, which was invented by Hoyle/Wickramasinghe, and is now an observational fact, documented in the *Journal of Cosmology* in numerous papers and figures not in the above reference list. The *Journal of Bacteriology* also suffers from an Astrobiological deficiency, as shown in journalofcosmology.com.

Biological information is important to cosmology, just as cosmology is important to biology. According to the standard LCDMHC cosmology, the first stars were formed by the collisionless fluid mechanics of gas and dust and the first planets were formed in small numbers after stars appeared: both after 300,000,000 years not 300,000 years predicted by HGD cosmology. Only a few planets can form per star from LCDMHC cosmology, under conditions that make life formation anywhere at any time virtually impossible. HGD cosmology predicts 30,000,000 planets exist per star, not eight or ten usually assumed, and that life formation is inevitable in the DNA-RNA form we observe on Earth. Observations confirm the millions of planets per star, falsifying the collisionless fluid mechanical basis of LCDMHC cosmology. Many forms of extraterrestrial life have been observed, taken from water extracted from fresh meteorites, including living marine diatoms.

