Halophilic archaea on Earth and in space: growth and survival under extreme conditions

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Salts are abundant on Mars, and any liquid water that is present or may have been present on the planet is expected to be hypersaline. Halophilic archaea (family Halobacteraeaceae) are the microorganisms best adapted to life at extremes of salinity on Earth. This paper reviews the properties of the Halobacteriaceae that may make the group good candidates for life also on Mars. Many species resist high UV and gamma radiation levels; one species has survived exposure to vacuum and radiation during a space flight; and there is at least one psychrotolerant species. Halophilic archaea may survive for millions of years within brine inclusions in salt crystals. Many species have different modes of anaerobic metabolism, and some can use light as an energy source using the light-driven proton pump bacteriorhodopsin. They are also highly tolerant to perchlorate, recently shown to be present in Martian soils, and some species can even use perchlorate as an electron acceptor to support anaerobic growth. The presence of characteristic carotenoid pigments (α-bacterioruberin and derivatives) makes the Halobacteriaceae easy to identify by Raman spectroscopy. Thus, if present on Mars, such organisms may be detected by Raman instrumentation planned to explore Mars during the upcoming ExoMars mission.

1. Introduction

The ExoMars mission to Mars, planned to be launched in 2018, will include Raman spectroscopy equipment to probe the chemical properties of the environment near the landing site [1]. As Raman spectroscopy is a
sensitive technique for the detection of certain biological molecules such as carotenoid pigments, the ExoMars mission will provide new possibilities to search for specific biomarkers as an indication of present or past life on Mars.

If life exists today or has existed on Mars in earlier periods when conditions on the planet were more conductive to life, it is tempting to speculate that such life was based on salt-requiring and/or salt-tolerant microorganisms. Life depends on the presence of liquid water, and liquid water can exist at the temperatures prevailing on Mars only when the freezing temperature becomes sufficiently depressed by dissolved salts. It is becoming increasingly clear that chloride and sulfate salts are abundantly present on Mars. A search for halophilic microorganisms may therefore be a major goal in the quest for the discovery of life on Mars, now or in the past.

Life in hypersaline environments on Earth is extremely diverse, as many different types of microorganisms (and a few macroorganisms as well) are adapted to exist in brines, often even up to NaCl saturation. The halophiles par excellence are the members of the family Halobacteraeaceae, which belongs to the Euryarchaeota phylum of the archaea. They are by no means the only microorganisms able to survive and even to thrive at the highest salinities, but they are the most abundant ones and also the most versatile ones. Moreover, they contain specific carotenoids and other molecules that can be used as biomarkers that can be detected with great sensitivity using Raman spectroscopy.

This review paper first summarizes the evidence for the existence of salts and brines on Mars, and then explores different hypersaline environments on Earth as possible analogues that simulate the Martian environment to different extents as a potential biotope. The properties of the halophilic archaea family Halobacteriaceae are then presented with special emphasis on those features that may be relevant for survival and even growth on Mars. Finally, the applications of Raman spectroscopy for the detection of halophilic archaea, and some other halophilic microorganisms as well, in extreme environments on Earth and in laboratory simulation experiments are discussed in view of the possibility to search for similar biomarkers by the ExoMars Lander in the near future.

2. Salts and brines on Mars

It has been stated that neither liquid brines nor liquid water are currently stable on Mars [2,3]. Still, liquid brines could be temporarily present in certain areas, and the existence of stable brines in the Martian subsurface cannot be excluded [2]. There is even some evidence for the presence of liquid water at shallow depths beneath the surface [4].

The observation of ‘recurring slope lineae’, what looks like seasonal flows on (relatively) warm Martian slopes as observed by the Mars Reconnaissance Orbiter [5], revived the discussion about the possible availability of liquid brines on present-day Mars. Temperatures on these slopes during the warm season can be as high as 250–300 K. It was calculated that the observed seasonality of the apparent flows on these slopes could be best explained by a solution with a freezing temperature of approximately 223 K (approx. −50°C) [6].

Salts are considered to be an important component of the fine-grained regolith on Mars, with (Mg,Na)SO₄, NaCl and other chlorides, and (Mg,Ca)CO₃ as likely components [7]. The presence of hygroscopic salts such as NaCl, CaCl₂ and MgCl₂ may also lead to the formation of droplets of liquid brine, at least locally and temporarily, as a result of deliquescence [8,9].

Another, only recently recognized component of the salts on Mars is perchlorate. The source of the formation of these perchlorate salts and the implications of their presence for the possibility of life on the planet are discussed in §4e. Perchlorate salts of sodium and magnesium are highly hygroscopic, and they can absorb moisture and form approximately 1 mm large liquid spheroids at temperatures as low as 225 K [10], thus increasing the availability of liquid water at least locally.
3. Model systems on Earth for the study of life on Mars

Space exploration programmes, including the search for life on Mars and other planets, have greatly benefited from the exploration of the most extreme environments on Earth as model systems, simulating at least to some extent the kind of conditions to which microorganisms on Mars may be exposed. Terrestrial model systems were selected to represent different combinations of extreme conditions: wet and hypersaline, based on dominance of NaCl, CaSO₄ or MgSO₄ (thus including both kosmotropic-stabilizing and chaotropic-destabilizing ions [11]), dry and hypersaline, cold and hypersaline, and acidic and hypersaline.

Wet and hypersaline NaCl-based extreme environments used as model systems for the study of life on Mars include continental saline environments such as evaporites of gypsum, halite and other materials in the alkaline Wadi Natrun, Egypt, the Salar de Atacama and other evaporite sites in the Atacama Desert, and Tunisian sabkhas [12,13]. The study of endoevaporitic cyanobacteria and other microorganisms within gypsum and halite evaporites in the lagoons of Baja California, Mexico [14] also belongs to this category. Permian (260 Ma) and Miocene (6 Ma) gypsum-permineralized microfossils from New Mexico and Italy, respectively, as well as recent gypsum deposits from Australia, Mexico and Peru containing microfossils of diatoms, cyanobacteria and other microorganisms were also studied as model systems for the search for life on Mars. These studies included Raman spectroscopy among the methods used [15].

As Martian soils may contain magnesium sulfate as the dominant salt, it is also interesting to explore life in terrestrial environments rich in epsomite (MgSO₄·7H₂O). One of these environments is the soil of the Great Salt Plains of Oklahoma. About one-third of the isolates collected from the site grew in the presence of 2 M MgSO₄, and these included representatives of the bacterial genera \textit{Halomonas}, \textit{Staphylococcus} and \textit{Halobacillus} [16]. No epsomite-tolerant members of the archaea were retrieved during this study. Another magnesium-sulfate-rich environment is Hot Lake, WA, USA, which recently became an object of study once more, after having been neglected for many years. A molecular and phenetic characterization of the bacterial assemblage of Hot Lake was made in view of its potential importance as a model system for life on Mars. All isolates able to grow in the presence of 1.7 M NaCl or 2 M MgSO₄ belonged to the bacteria, and included both Gram-positive and Gram-negative types. The most frequently cultured species belonged to the genera \textit{Halomonas}, \textit{Idiomarina}, \textit{Marinobacter}, \textit{Marinococcus}, \textit{Nesterenkonia}, \textit{Nocardiopsis} and \textit{Planococcus}. No archaea were isolated during this study, and a parallel culture-independent, 16S rRNA gene-based study showed very low abundance of archaea [17].

The possibility for halophilic life to persist under conditions of extreme aridity was extensively investigated in the Atacama Desert, Chile, which is one of the driest places on our planet. In certain sites, halite evaporites are found, which are inhabited by unicellular cyanobacteria morphologically similar to \textit{Chroococcidiopsis}, but phylogenetically closer to \textit{Halothecae} [18]. Further Atacama Desert studies, including the use of Raman spectroscopy to search for specific biomarkers, are discussed in §5.

In view of the low temperatures prevailing on Mars, investigations of the microbiology of cold and hypersaline environments on Earth are also of interest. However, only very few such studies have been performed thus far. There exist a few hypersaline lakes in Antarctica. One of these, Deep Lake located in the Vestfold Hills, is the source of \textit{Halorubrum lacusprofundi} [19], an archaeon whose properties are described in §4d. A recent study of the brines of an ice-sealed Antarctic lake (Lake Vida, Victoria Valley, East Antarctica) with a salinity of 200‰ showed presence of a diverse community of bacteria displaying a low level of radiolabelled leucine at the \textit{in situ} temperature of −13°C [20].

The possibility that some Martian soils may not only be saline, but also acidic makes it worthwhile to search for analogous salty and acidic sites on our planet. Such sites are very rare. There are a number of small ephemeral acidic and hypersaline lakes in Western Australia that can serve as model systems for Mars-related studies [21]. The most acidic of these lakes included in a survey had pH values of 2.8, 3.0, 4.2 and 4.3, with 83, 160, 214 and 40 ppt total dissolved
salts, respectively. Using bacteria-specific primers, a number of 16S rRNA sequences were retrieved from these lakes, belonging to different groups: mainly Proteobacteria, Bacteroidetes and Actinobacteria [22]. No reports exist yet on the isolation of specialized acidophilic halophiles from these lakes. Another saline and acidic environment suggested as a suitable Mars analogue is the endolithic community existing in the acidic salt (natrojarosite) deposits of Rio Tinto, Spain, whose waters have a pH of approximately 1.0. In a culture-independent molecular study, small subunit rRNA sequences of both prokaryotic and eukaryotic phototrophic microorganisms were retrieved from these acidic salt deposits [23].

4. The Halobacteriaceae as model organisms and the properties that may make them suitable for life on Mars

Halophilic life on planet Earth is extremely diverse. We find microorganisms adapted to life at high salt concentrations, even up to NaCl saturation, in all three domains of life: archaea, bacteria and eukarya. Functionally, halophiles can perform nearly all processes known at low salt concentrations: oxygenic and anoxygenic photosynthesis, respiration using molecular oxygen or other electron acceptors such as nitrate and sulfate, fermentation and chemosynthesis [24]. To osmotically adjust the intracellular environment with the external medium, different strategies have evolved. One strategy is based on the accumulation of high concentrations of K+ and Cl− ions within the cell. This option is used by a few groups only: the Halobacteriaceae (archaea) and Salinibacter, an extremely halophilic member of the Bacteroidetes (bacteria) found in the same habitats where members of the Halobacteriaceae thrive. This ‘salt-in’ strategy requires a far-going adaptation of the intracellular enzymatic machinery to the constant presence of molar concentrations of KCl. A different, and far more widespread strategy, used by most other groups of halophilic and halotolerant microorganisms, is the biosynthesis and/or accumulation from the environment of simple, generally electroneutral organic ‘compatible’ solutes used to provide the necessary osmotic balance [24,25].

This review explores the suitability of archaea of the family Halobacteriaceae as candidates to grow and/or survive on Mars, now or in the past. The family contains those microorganisms growing up to the highest salt concentrations and better adapted to life at high salt than any other known microbial group. As of February 2014, the family was composed of 47 genera with a total of 165 species. Basically, the Halobacteriaceae are aerobic chemoheterotrophic microorganisms that obtain their energy by the oxidation of simple organic compounds using molecular oxygen as the electron acceptor for respiration [26]. At first sight, organisms with such a mode of metabolism are not very promising candidates for extraterrestrial life. However, many of the halophilic archaea that inhabit the hypersaline brines worldwide have a surprising metabolic versatility, and/or have unusual properties enabling their survival and possibly even growth under the kind of conditions prevailing on Mars. These extreme halophiles are therefore excellent models for astrobiology studies, even to the extent that DasSarma termed these organisms as ‘exophiles’ [27]. Their importance for the study of life on Mars has been realized since the last years of the twentieth century [28].

The following paragraphs summarize the different properties that make the Halobacteriaceae relevant model organisms for studies on life on Mars and also possibly elsewhere in the universe.

(a) Diverse modes of energy generation in the absence of molecular oxygen

Although the Halobacteriaceae generally grow as aerobic heterotrophs, many members of the family possess different modes of energy generation and even anaerobic growth under anaerobic conditions [29,30]. One such way is the use of light energy instead of chemical energy obtained by aerobic respiration. Many members of the family can produce the membrane-bound purple protein bacteriorhodopsin, a 25 kDa protein that carries a retinal moiety bound as a Schiff base to one of its lysine residues. Bacteriorhodopsin acts as a light-driven proton pump. Following
absorption of a photon (maximum absorbance at 570 nm), the molecule undergoes a complex photocycle during which the Schiff base is sequentially protonated and deprotonated. Protons are taken up from the cytoplasm, and during deprotonation released to the external medium. A proton gradient is thus established, which can be used to drive the formation of adenosine triphosphate. Anaerobic light-driven growth is thus possible, but organic substrates are still needed as carbon source [29]. Photoautotrophic growth is not possible.

Many members of the Halobacteriaceae can replace oxygen with alternative electron acceptors for respiration. The ability to reduce nitrate and grow by denitrification is widespread within the group. Other electron acceptors supporting growth by anaerobic respiration in many species are trimethylamine N-oxide, dimethylsulfoxide, fumarate and even perchlorate and chlorate (see §4e). Fermentative growth is possible in a few species of Halobacteriaceae only. Members of the genus *Halobacterium*, but as far as is known not of any of the other genera of the family, can grow anaerobically by fermentation of the amino acid arginine [29]. One species, *Halorhabdus tiamatea*, a non-pigmented extremely halophilic archaeon isolated from Shaban Deep, a deep-sea, hypersaline anoxic basin on the bottom of the Red Sea, prefers an anaerobic or microaerophilic lifestyle over respiration with oxygen [31]. The mode of fermentation used by this species is not yet clear, but analysis of its genome showed presence of an l-lactate dehydrogenase, which may participate in the process [32].

(b) Survival in the space environment exposed to high radiation and high vacuum

A halophilic archaeon isolated from evaporites in Baja California, Mexico and originally designated ‘Haloarcula isolate G’ was exposed to high radiation in space. It was dried on quartz discs and flown for 15 days in orbit in 1994 in the BIOPAN facility, a small retrievable capsule developed by the European Space Agency for exposure of biological samples to conditions of outer space. After exposure to $10^4$ kJ m$^{-2}$ radiation in high vacuum, part of the cells (about one out of $10^7$–$10^8$ cells, when compared with a laboratory control experiment) had retained its viability, as shown by colony counts on agar plates and growth in dilutions in liquid culture [33]. A comparative study using the archaeal ‘Haloarcula isolate G’ and *Halobacterium salinarum* NRC-1 and different non-halophiles (*Deinococcus radiodurans*, *Escherichia coli*, *Pseudomonas fluorescens*), testing survival after desiccation and subsequent exposure to $-20$ or $-80^\circ$C, showed that the halophiles survived better under dry and low-temperature conditions than the non-halophiles. The two halophilic archaea survived desiccation and 10 freeze–thaw cycles at $-20$ and $-80^\circ$C for at least 144 days, whereas *E. coli* and *P. fluorescens* did not survive any of the treatments [34]. A later taxonomic study identified ‘isolate G’ not as a member of the genus *Haloarcula*, but instead belonging to the genus *Halorubrum*. Together with similar strains obtained from Western Australia and from Naxos, Greece, it was described as a new species, *Halorubrum chaoviator*, ‘the traveller of the void’, referring to the exposure of the type strain to conditions of outer space [35].

The 1994 BIOPAN experiment was not the only occasion at which members of the Halobacteriaceae were sent into space. There have been at least two other missions that included survival experiments with halophilic archaea. During the EXPOSE-E mission on the Space Shuttle Atlantis, launched in February 2008, *Halococcus dombrowskii* was exposed for 559 days to the space environment, and *Halorubrum chaoviator* was sent once more into space in November 2008 with the EXPOSE-R mission with the Russian Progress 31-P [36]. Unfortunately, no results of these experiments have yet been published.

There have been numerous studies to assess the resistance of halophilic archaea to UV radiation, and some of these were specifically intended to investigate the possibility for such organism to survive on Mars. Cells of *H. dombrowskii*, an isolate obtained from a salt mine [37], and other members of the family (*Hbt. salinarum* NRC-1, *Haloarcula japonica*) embedded in a thin layer of laboratory-grown halite showed no loss of viability (assessed using the LIVE/DEAD kit dyes) after exposure to 21 kJ m$^{-2}$, and they resumed growth in liquid medium with lag phases of 12 days or more after exposure to 148 kJ m$^{-2}$ of radiation supplied by a ‘Mars UV simulator lamp’. The $D_{37}$ (dose of 37% survival) was greater than or equal to 400 kJ m$^{-2}$. However, in liquid culture,
the $D_{37}$ was only approximately 1 kJ m$^{-2}$ [38]. A comparison of the survival of the haloarchaea *Natrialba magadii* (an alkaliphilic species) and *Haloferax volcanii* exposed to vacuum UV radiation with the radiation-resistant bacterium *Deinococcus* showed that *Nab. magadii* survived exposure to high vacuum for 1 h better than *Deinococcus*. Following doses of synchrotron radiation up to 150 J m$^{-2}$ (57.6–200 nm), the two archaea survived as well as *Deinococcus*, but at higher doses, the archaea performed less well [39,40].

One of the factors that may contribute to the high degree of resistance of halophilic archaea to high levels of ionizing radiation, in addition to the presence of multiple copies of the genome within each cell, is the high intracellular concentration of halide ions (chloride, possibly bromide as well) that act as ‘chemical chaperones’. The intracellular salts may protect the cells against radiation damage by scavenging reactive oxygen species, as shown in a study with *Hbt. salinarum* NRC-1 [41].

A number of research efforts have targeted the mechanisms and the enzymes responsible for the UV resistance of Halobacteriaceae. When exposed to UV-C (254 nm, up to 500 J m$^{-2}$), the nucleotide excision repair genes *uvrA*, *uvrB* and *uvrC* were upregulated in *Halococcus hamelinensis*, an isolate from living stromatolites in Shark Bay, Western Australia. When incubated in the light, a 20-fold increase in photolyase *phr2* was also observed [42].

The *uvr* genes and *phr2* were also identified as relevant to excision repair after UV-induced damage in *Hbt. salinarum* NRC-1, an organism that withstands up to 110 J m$^{-2}$ UV radiation [43]. Different repair mechanisms were identified in this organism: photoreactivation, d(CTAG) methylation-directed mismatch repair, four oxidative damage repair enzymes and two proteases for eliminating damaged proteins [44]. This organism is also highly resistant to desiccation, high vacuum and $^{60}$Co gamma radiation (5 kGy at least), and double-strand breaks in the DNA are efficiently repaired. Among the factors implicated in its radiation resistance are the carotenoid pigment bacterioruberin as well as the intracellular salts [45]. Mutant studies using *Hbt. salinarum* NRC-1 showed the homologous recombination protein Mre11 to be essential for recovery following UV-C exposure, but the Rad50 protein is not essential for the Mre11-dependent repair of DNA double-strand breaks [46].

Another factor that may contribute to the high radiation resistance of *Hbt. salinarum* NCR-1, and possibly of other members of the family as well, is the high intracellular concentration of manganese ions found in manganese–antioxidant complexes. Mutants resistant to increased doses of ionizing radiation (60% survival at 17 Gy, when compared with 5 Gy for the parent strain) contained increased intracellular concentrations of manganese (15–30 µM). However, deletion mutants lacking superoxide dismutase and catalase showed no decreased survival after exposure to ionizing radiation [47].

Not all members of the Halobacteriaceae, however, are equally resistant to exposure to UV radiation. *Natronorubrum* strain HG-1, an isolate from a hypersaline lake the Kulunda Steppe, Altai, Russia, cannot survive for more than a few hours in a simulated Martian environment with UV exposure similar to that at the surface of Mars, low temperatures (+4, −20, −80°C) and desiccation [48].

(c) Longevity of dormant cells of Halobacteriaceae entrapped within salt crystals

When halite crystals are formed during the evaporation of salt-saturated brines, brine inclusions often remain within the crystals. Halophilic archaea (and other microorganisms as well) that were present in the brine can thus become entrapped within the growing crystals. Many experiments in the past two decades have shown that members of the Halobacteriaceae can retain their viability within halite crystals for periods of thousands and possibly of millions of years [25,37]. Several species were isolated from underground salt deposits. One such isolate is *H. dombrowskii*, obtained from Permian salt from an Austrian salt mine and named in honour of Heinz Dombrowski who pioneered isolation of microorganisms from ancient material such as salt deposits in the 1960s [49]. Other examples are *Halococcus salifodinae*, also from Permian rock salt in Austria, and *Halosimplex carlsbadense* from Permian salt in New Mexico.
Many discussions have been devoted to the question whether indeed such isolates represent organisms that grew many million years ago and have remained viable in a dormant state since the time they became entrapped within halite crystals. It is not easy to prove that the organisms have not grown in situ more recently following local dissolution of some of the salt [50]. But evidence is accumulating that halophilic archaea may indeed survive for millions of years, and then can either be revived as colonies on agar plates or be identified based on 16S rRNA gene sequences. It is not possible to discuss all the published data within the framework of this brief review paper.

Recent studies of cores from Death Valley, CA containing layers of halite deposited 10–35 ka ago and from Saline Valley, CA (salt layers dated 75 and 150 ka) have greatly contributed to our insights into the longevity of halophilic archaea within salt crystals that serve as repositories for microbial life. Microscopic examination shows the prokaryotic cells entrapped in brine inclusions within the salt crystals to be smaller than their modern counterparts grown in nutrient-rich media [51]. Reduction in cell size is commonly found as a reaction to prolonged starvation. Still, it must be asked how such cells may have survived for so many years in the absence of a supply of organic carbon and energy sources. A partial answer to this question is that not only do cells of Halobacteriaceae become entrapped within the growing salt crystals, but also cells of the alga Dunaliella, generally found in salt-saturated brines together with the archaea. Dunaliella cells are large and contain huge amounts of glycerol produced to provide osmotic stabilization for this alga, which in contrast to the archaea excludes salt from its cytoplasm. The organic material of a single Dunaliella cell may suffice as a carbon and energy source to support survival of halophilic archaea for prolonged periods [52,53].

(d) Adaptation to low temperatures

Little is known about the growth and adaptation mechanisms of halophilic archaea to life at low temperatures. The best studied cold environment that harbours a community of Halobacteriaceae is Deep Lake, located in the Vestfold Hills, Antarctica. This hypersaline (210–280 g l⁻¹ salts) lake never freezes as a result of the high salinity, not even at the minimum recorded water temperature of −20°C. Water temperatures above 0°C are observed only in the top few metres during the summer months, and the highest measured temperature is +11.5°C. It was estimated that no more than six generations of archaea can develop annually at the in situ temperatures [54]. Halorubrum lacusprofundi was isolated from this lake. Its optimal growth is found at 31–37°C, but slow growth was still recorded at 4°C, a temperature too low for the other members of the family [19]. The ability to synthesize unsaturated diether lipids, which are otherwise seldom found in the archaea, may be one key adaptation of Hrr. lacusprofundi to life at low temperatures [55]. A recent metagenomic study of the archaean community inhabiting Deep Lake showed that the lake is populated, in addition to Hrr. lacusprofundi, by three other types of Halobacteriaceae: a Halohasta sp., a Halobacterium sp. and a type designated DL31, not belonging to any of the currently recognized genera. The complete genomes of all four types could be reconstructed from the metagenomic data [54], but except for Hrr. lacusprofundi no other cultured halophilic cold-tolerant haloarchaea have yet been described.

(e) Perchlorate reduction

The wet chemistry laboratory of the Phoenix Lander found high concentrations of perchlorate in Martian soils near its landing site: leachates of soil samples contained 0.4–0.6% perchlorate by mass. Martian soils are thus highly oxidizing [56]. The perchlorate is probably formed by atmospheric interactions between ozone and volatile chlorine compounds [57,58]. Perchlorate salts are highly deliquescent, and therefore they may promote the formation of droplets or films of liquid water, thereby enhancing the possibilities for life [57]. On the other hand, perchlorates are strong oxidizers, and their presence may be harmful to life forms.
However, perchlorate can be used as an electron acceptor for anaerobic respiration by some prokaryotes, bacteria as well as archaea. Early studies with anaerobic enrichment cultures in the presence of 5 mM sodium perchlorate and salt concentrations up to 70–110 g l\(^{-1}\) salt yielded growth with reduction of perchlorate [59]. The organisms that developed in these cultures were not further characterized. Pure cultures of the archaeon *Haloferax denitrificans* and the bacterium *Halomonas halodenitrificans* reduced perchlorate as well as nitrate [60]. Use of the halophilic archaeon *Haloferax mediterranei* was even proposed for the bioremediation of high-salt waste-water containing chlorate and perchlorate [61].

Halophilic archaea are able to tolerate far higher concentrations of perchlorate than the approximately 5 mM used in the above-described experiments. All Halobacteriaceae species tested grew well in NaCl-based media containing 0.4 M sodium perchlorate, and *Hfx. mediterranei* even grow weakly in the presence of 0.6 M. But, at the highest tolerated perchlorate concentrations, cells were swollen and distorted. The ability to use perchlorate as an electron acceptor for anaerobic respiration, supporting growth in the absence of molecular oxygen, was shown for several species (*Hfx. mediterranei*, *Hfx. denitrificans*, *Hfx. gibbonsii*, *Haloarcula marismortui*, *Har. vallismortis*). Species growing anaerobically on perchlorate generally could also use chlorate as an electron acceptor [62]. These results show that, rather than being toxic, presence of perchlorate can be favourable for the development of halophilic archaea in the absence of molecular oxygen, provided that a suitable electron donor and energy source is also available.

5. Raman spectroscopy as a tool to detect halophilic archaea and other halophilic microorganisms

The first pioneering studies of the detection of carotenoids of members of the Halobacteriaceae by resonance Raman spectroscopy were published by Marshall et al. [63]. In view of the finding of halite and sulfate evaporites on Mars, they provided the first Raman spectra of bacterioruberin carotenoids and of bacteriorhodopsin, using *Hbt. salinarum*, *Halococcus morrhuae* and *Natrinema pallidum* as model organisms. To test the suitability of Raman spectroscopy for the detection of extremely halophilic archaea embedded in halite in terrestrial and possibly extraterrestrial samples as well, Fendrihan et al. [64] embedded different species of Halobacteriaceae in growing halite crystals. Strong bands of carotenoids (bacterioruberin) were detected as well as signals derived from peptide bonds and nucleic acids. Raman spectroscopy was also used to probe colonies of *Hbt. salinarum* on agar plates. Pigmentation was found not to be uniform, but instead, rings of denser and less dense pigmentation were observed [65].

A detailed study of the Raman bands of bacterioruberin was made, combined with a study of the Raman spectrum of salinixanthin, the carotenoid acyl glycoside of *Salinibacter*, the extremely halophilic representative of the bacteria often found in the same salt-saturated habitat in which members of the Halobacteriaceae thrive [66]. Small, hand-held Raman spectrometers have also been successfully tested for the detection of these pigments [67].

Raman spectroscopy can sensitively detect other biomarkers in high-salt, low water activity environments as well. Examples are chlorophyll *a*, different carotenoids and scytomin in colonies of cyanobacteria (*Gloeocapsa, Nostoc*) in gypsum and other evaporites in the High Arctic [68] and within halite from hyperarid areas of the Atacama Desert, Chile [69]. Here, the use of hand-held instruments in the field was combined with a Raman microscope to obtain high-resolution measurements in the laboratory. Both hand-held and laboratory-size instruments were used to probe the coloured layers of different types of cyanobacteria and of purple sulfur bacteria within the gypsum crust that develops on the bottom of saltern evaporation ponds in Eilat, Israel, at a salinity of approximately 200 g l\(^{-1}\) [67].

Pigments are not the only possible biomarkers to indicate presence of halophilic and other microorganisms using Raman spectroscopy. Simple organic biomolecules such as amino acids (glycine, alanine, serine) trapped within fluid inclusions inside halite crystals can be detected [70]. Most halophilic and halotolerant microorganisms (but not including members of
the Halobacteriaceae and Salinibacter) accumulate high concentrations of different organic osmotic solutes to provide osmotic balance of the cells with the hypersaline environment. Such organic osmotic solutes can also be excellent biomarkers. Therefore, a library of Raman spectra was made of the best-known osmotic solutes [71], and techniques were developed for the detection of such solutes in different halophilic bacteria. For the reliable detection of the compounds, extraction and some purification were necessary; direct detection of these compounds within cells or cell pellets proved problematic [72].

A recent review on the use of Raman spectroscopy in microbiology [73] discusses many of the above-mentioned aspects: the search for pigments in evaporites, detection of pigmented organisms in salt lakes and saltern evaporation ponds, and the characterization of organic osmotic solutes.

6. Final comments

Whether organisms similar to the halophilic archaea of the family Halobacteriaceae indeed live on Mars, or may have lived there in earlier times when the climate there was more conducive to life, is still unknown. As shown above, they make good candidates for organisms that can adapt to the Martian environment. It has been speculated before whether recent or ancient halobacteria may be present entrapped within salt on Mars [74].

Raman spectroscopy is an excellent technique to detect halophilic archaea, thanks to the presence of specific carotenoids and other potential biomarkers such as the retinal protein bacteriorhodopsin. NaCl does not have Raman signals, so biomarkers entrapped within halite crystals can be detected without interference by the crystal matrix.

A Raman spectrometer will be included in the instrumentation planned to land on Mars in 2018 with the ExoMars mission, and therefore different model geological and biogeological specimens have been tested as reference material, including evaporites colonized by carotenoid-containing microorganisms [1]. We will have to wait and see whether or not the ExoMars Lander, if indeed it will successfully land on Mars, will detect similar biomarkers there. If it will, then our current speculations about possible present-day or past halophilic life on that planet may well become confirmed by solid data.

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