



# Extremophilic models for astrobiology: haloarchaeal survival strategies and pigments for remote sensing

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## Abstract

Recent progress in extremophile biology, exploration of planetary bodies in the solar system, and the detection and characterization of extrasolar planets are leading to new insights in the field of astrobiology and possible distribution of life in the universe. Among the many extremophiles on Earth, the halophilic Archaea (Haloarchaea) are especially attractive models for astrobiology, being evolutionarily ancient and physiologically versatile, potentially surviving in a variety of planetary environments and with relevance for in situ life detection. Haloarchaea are polyextremophilic with tolerance of saturating salinity, anaerobic conditions, high levels of ultraviolet and ionizing radiation, subzero temperatures, desiccation, and toxic ions. Haloarchaea survive launches into Earth's stratosphere encountering conditions similar to those found on the surface of Mars. Studies of their unique proteins are revealing mechanisms permitting activity and function in high ionic strength, perchlorates, and subzero temperatures. Haloarchaea also produce spectacular blooms visible from space due to synthesis of red–orange isoprenoid carotenoids used for photoprotection and photorepair processes and purple retinal chromoproteins for phototrophy and phototaxis. Remote sensing using visible and infrared spectroscopy has shown that haloarchaeal pigments exhibit both a discernable peak of absorption and a reflective “green edge”. Since the pigments produce remotely detectable features, they may influence the spectrum from an inhabited exoplanet imaged by a future large space-based telescope. In this review, we focus primarily on studies of two Haloarchaea, *Halobacterium* sp. NRC-1 and *Halorubrum lacusprofundi*.

**Keywords** Astrobiology · Biosignature · Haloarchaea · Phototrophy · Purple membrane · Retinal · Polyextremophile

## Abbreviations

UV	Ultraviolet
LUCA	Last universal common ancestor
pI	Isoelectric point
HOG	Haloarchaeal orthologous group
BR	Bacteriorhodopsin

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## Introduction

The emerging field of astrobiology addresses a fundamental philosophical question: ‘Are we alone in the universe?’ (Morrison 2001). Among the nearly 200 planets and satellites in the solar system, and ~4000 planets discovered so far in our galaxy, Earth remains the only known living world that can enlighten us about the potential answer (Lissauer et al. 2014; Walker et al. 2018). While common life forms on Earth have traditionally informed us about the limits and capabilities of living organisms, the discovery of an ever-increasing variety of extremophiles over the past 50 years has expanded our appreciation of the physiological and biochemical capabilities of living organisms as well as illuminating the long history of evolution on our planet. Of special interest are extremophiles with the potential to survive in a variety of planetary environments within the solar system as well as the galaxy, which are relevant for in situ life detection.

Earth's extremophiles may be divided into two distinct categories: specialists, adapted to a single extreme; and generalists, adapted to multiple extremes (Rothschild and Mancinelli 2001). While considerable effort has gone into studies of specialists, such as hyperthermophiles and anaerobes, generalists like the halophilic Archaea (Haloarchaea) remain of significant interest for astrobiology since they survive multiple stressors, like those on the surface of Mars and potentially habitable exoplanets. Haloarchaea tolerate multiple extremes, forming dense cultures, and harbor light-capture pigments and phototrophic metabolism, which are potentially remotely detectable (DasSarma 2006). Consequently, Haloarchaea may influence the spectrum from an inhabited exoplanet, detectable from a future space telescope (Schwieterman et al. 2018; DasSarma and Schwieterman 2019).

Many Haloarchaea are facultative aerobes with the ability to grow with or without oxygen, utilizing a variety of terminal electron acceptors, and in addition they have the capacity for phototrophic growth, making them physiologically unusually versatile microorganisms (DasSarma et al. 2012; Kilic et al. 2017; Mancinelli and Hochstein 1986; Martínez-Espinoza et al. 2014; Müller and DasSarma 2005; Sorokin et al. 2019; Sumper et al. 1976). Haloarchaea also are highly pigmented due to synthesis of carotenoids, resulting in bright blooms in hypersaline brine which may appear red, orange, or purple in color (DasSarma and Schwieterman 2019; de la Vega et al. 2016; Rodrigo-Baños et al. 2015; Yim et al. 2015). Coupled with our ability to isolate, construct, and characterize variants and mutants in the laboratory perturbed in their growth, pigmentation, and extremophilic properties, Haloarchaea offer the prospect of deeper insights into some fundamental questions in astrobiology.

## Evolution and habitability

Our understanding of life's evolution on Earth is founded on knowledge of a common descent for all extant life, a concept supported by the existence of a universal genetic code (Söll and RajBhandary 2006). The ancestral life form from which all known life evolved is referred to as the last universal common ancestor (LUCA). Geochemical evidence of fossilized microorganisms in stromatolites suggests that LUCA appeared 3.7–3.8 billion years ago, when physical conditions were quite 'extreme' compared to conditions on the surface of Earth today (Fenchel 2002). As a result, the possibility that certain extremophilic species, e.g., early evolving Archaea, may resemble these early life forms has been postulated. However, extant species which are available for experimentation must have undergone considerable evolutionary changes during the billion years-long history of the planet and are likely to be quite different, as a result

of processes such as lateral gene transfers (Kennedy et al. 2001; Koonin 2015).

Recent studies of diverse extremophiles and their extreme environments on our planet nevertheless have offered the opportunity for better understanding the characteristics of life forms likely important for success on the early Earth (Merino et al. 2019). They are also leading to insights into the limits to life at extremes of temperature, pressure, humidity, radiation and toxins. Extreme environments on Earth offer terrestrial analogs for understanding potential habitability of other planetary bodies. The ideal terrestrial microbial models for astrobiology should be capable of tolerating multiple extremes, like the Haloarchaea, which have evolved to survive in underground evaporites, as well as submarine brine pools beneath the sea, and in the upper fringes of the stratosphere above the protective ozone layer (DasSarma and DasSarma 2017; 2018; DasSarma et al. 2019; Grant et al. 1998; Landis 2001).

## Models for astrobiology

Among the great diversity of extremophiles known, Haloarchaea represent excellent models for astrobiology (DasSarma 2006, 2007; DasSarma and DasSarma 2017). On Earth, they are frequently observed in buoyant, planktonic blooms above photosynthetic mats, which are similar to stratified microbial communities in ancient fossilized stromatolites (Noffke et al. 2013). Haloarchaeal blooms are striking in color and offer the opportunity for direct visualization by remote sensing (Dalton et al. 2009). These ancient microorganisms survive exposure to a variety of extreme conditions found on Mars, including desiccation, radiation, subzero temperatures, and the presence of perchlorate oxidizers (Table 1). They have also been reported to survive launches into the stratosphere and exposure to space conditions (DasSarma and DasSarma 2018; Horneck et al. 2010).

Haloarchaea are members of the Domain Archaea, which likely evolved very early on Earth, possibly in the early Archaean (Fenchel 2002; DasSarma and Schwieterman 2019). The notion that Archaea appeared during the first billion years after formation of the planet is supported by the geochemical detection of isoprenoid compound derivatives used in their membrane lipids and pigments in ancient sediments. For example, such compounds have been detected in Isua, West Greenland sediments and dated to 3.7–3.8 billion years ago (Hahn and Haug 1986). The phototrophic and anaerobic metabolic capabilities of some Haloarchaea together with evidence for relatively recent acquisition of aerobic respiration by lateral gene transfer is consistent with such a scenario (Kennedy et al. 2001; Koonin 2015).

Success of Haloarchaea on the Earth's surface in modern times is highlighted by highly pigmented blooms in widely distributed hypersaline solar salterns and lakes. Some

**Table 1** Examples of extreme conditions tolerated by Haloarchaea

Extreme	Species	Environment
Salinity	<i>Halobacterium</i> species	Grows in saturated (> 30%) NaCl, e.g., in saltern crystallizers
Cold	<i>Halorubrum lacusprofundi</i>	Grows at low perennial temperatures to − 18 °C, e.g., in Deep Lake, Antarctica
Heat	<i>Halorhabdus tiamatea</i>	Grows at elevated temperatures to 60 °C, e.g. in deep sea brine pool, Red Sea
Oxygen-free	<i>Halobacterium</i> and <i>Haloferax</i> species	Grow anaerobically with alternate electron acceptors, e.g., nitrate, perchlorate
Desiccation	<i>Halobacterium</i> species	Survives in subsurface brine inclusions, e.g., in Permian salt deposits
Ionizing radiation	<i>Halobacterium</i> species	Survives ionizing radiation 8–11 kGy from LINAC and X-rays in laboratory
UV radiation	<i>Halobacterium</i> species	Survives exposure to 70 Jm <sup>−2</sup> UV–C radiation, e.g., in stratosphere
Low pressure	<i>Halorubrum lacusprofundi</i> , <i>Haloarcula</i> ‘G’	Tolerant of extremely low pressures, in stratosphere and space
Toxic ions	<i>Halobacterium</i> species	Tolerant of arsenic, magnesium, and perchlorate
Alkalinity	<i>Natronomonas pharaonis</i>	Grows at alkaline pH (8.5–9.0), e.g., in Lake Magadi, Kenya
Acidity	<i>Halarchaeum acidiphilum</i>	Grows at acidic pH (4.0–6.0), isolated from commercial salt

haloarchaeal blooms, such as in south San Francisco Bay salterns or Great Salt Lake, Utah, have been observed by remote sensing from visible and infrared imaging sensors on airplanes (Dalton et al. 2009) and visually by astronauts on the International Space Station (DasSarma et al. 2016) (<https://eol.jsc.nasa.gov/>). The early evolution of retinal pigments has also been proposed to account for a “Purple Earth” (DasSarma and Schwieterman 2019). The combination of polyextremophily, detectability, and ancient evolution of Haloarchaea makes them attractive as astrobiological models among the extremophiles.

## Two Haloarchaeal models

Two Haloarchaea have been extensively studied as astrobiology models, *Halobacterium* sp. NRC-1, a highly pigmented buoyant species, isolated from the solar salterns of San Francisco Bay, California, USA, and *H. lacusprofundi*, a more brightly pigmented, biofilm-forming species, isolated from the hypersaline Deep Lake, Antarctica (Anderson et al. 2016; DasSarma 2007; DasSarma et al. 2019; Reid et al. 2006). *Halobacterium* sp. NRC-1 is also distinguished by being the first among the Haloarchaea to have its genome sequenced (in 2000), an achievement that was followed by detailed postgenomic studies and characterization of the basis of survival under multiple extreme conditions (DasSarma 2004, 2006; Kennedy et al. 2001; Ng et al. 1998, 2000; Peck et al. 2000).

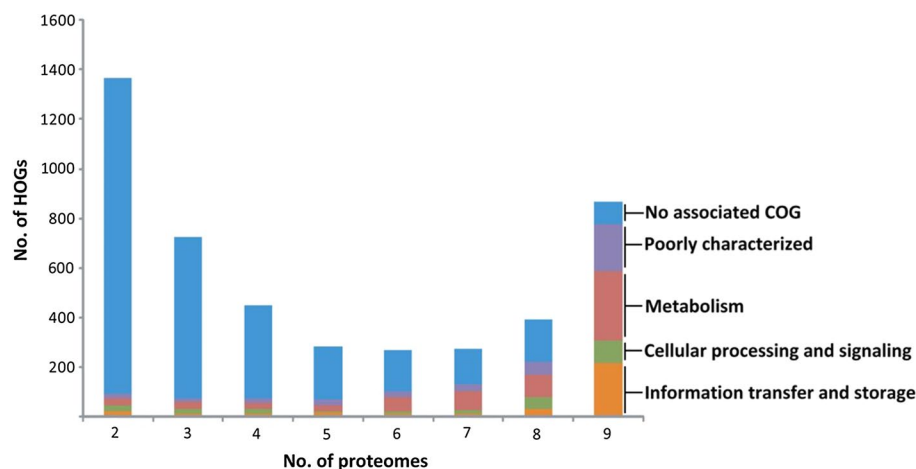
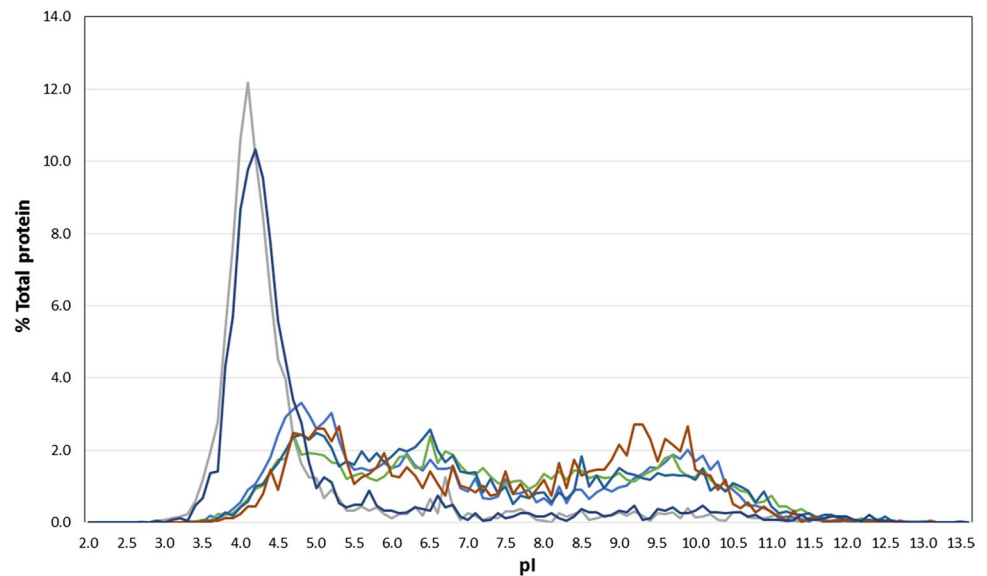
A series of whole-genome transcriptomic studies on *Halobacterium* sp. NRC-1 addressed mechanisms of tolerance to high and low osmolarity, elevated and reduced temperatures, and UV and ionizing radiation (Coker et al. 2007; Karan et al. 2013, 2014; McCready et al. 2005). Genetic and transcriptomic studies explored the capability of the species to tolerate and grow microaerobically and anaerobically, phototrophically, and with heavy metal ions (DasSarma et al.

2012; Müller and DasSarma 2005; Wang et al. 2004). A number of defining molecular characteristics were discovered, including core and signature genes, negatively charged proteins, expanded families of general transcription factors, and multiple origins and origin binding proteins (Capes et al. 2011, 2012). The *Halobacterium* sp. NRC-1 experimental system has provided considerable insights into the polyextremophilic characteristics of Haloarchaea (Figs. 1 and 2) (DasSarma et al. 2006).

Several recent studies compared the mesophilic *Halobacterium* sp. NRC-1 to the cold-adapted *H. lacusprofundi* for tolerance to multiple stressors (Anderson et al. 2016; DasSarma et al. 2013; Reid et al. 2006). Notable among them were survival following launches up to 35 km high in the atmosphere (DasSarma et al. 2017). The cold-adapted species was better able to survive and grow after stratospheric launches and these properties reflected better survival with freezing and thawing in the laboratory, and correlated to its more robust cold temperature response at genomic and transcriptomic levels.

An additional comparative study addressed the effects of exposure of these Haloarchaea to perchlorate, an oxidizing ion that is common on the surface of Mars (Laye and DasSarma 2018). Interestingly, *H. lacusprofundi* was able to grow anaerobically using perchlorate as a terminal electron acceptor, providing a metabolism for potential survival on the red planet. In addition, *H. lacusprofundi*  $\beta$ -galactosidase enzyme was overexpressed and purified from *Halobacterium* sp. NRC-1 and found to function with either high concentrations of sodium or magnesium perchlorate, as well as allowing biological catalysis under conditions reported on the surface of Mars (Laye and DasSarma 2018).

**Fig. 1** Isoelectric point distribution of haloarchaeal and non-haloarchaeal proteins. Percent of total proteins with pI values are plotted versus pI for the Haloarchaea, *Halobacterium* sp. NRC-1 (gray) and *Halorubrum lacusprofundi* (dark blue), and non-halophiles, *E. coli* (medium blue), *Bacillus subtilis* (light blue), *Methanocaldococcus jannaschii* (red) and *Schizosaccharomyces pombe* (green) (DasSarma and DasSarma 2015; Kennedy et al. 2001)



**Fig. 2** Distribution of haloarchaeal orthologous groups. Haloarchaeal orthologous groups (HOGs) identified by reciprocal BLAST analysis exhibit a bimodal distribution, with the largest number of proteins with unknown function and no associated Clusters of Orthologous

Groups (COGs) shown in blue. Those with known or predictable function present in haloarchaeal proteomes are shown in other colors (orange, green, reddish brown, and purple) with functions indicated on the right (Capes et al. 2012)

## Acidic proteins

Genomic analysis showed that *Halobacterium* sp. NRC-1, *H. lacusprofundi*, and other Haloarchaea have highly acidic proteomes (DasSarma and DasSarma 2015; Kennedy et al. 2001) (Fig. 1). In contrast, nearly all other organisms have a near neutral proteome, with both acidic and basic proteins present. This difference likely reflects the unique adaptive strategy Haloarchaea use in their high salt environment, maintaining osmotic equilibrium with high internal salt concentrations, and balancing the sodium ions in their environment with potassium (DasSarma and DasSarma 2017). This “salt-in” strategy requires that haloarchaeal proteins are able

to successfully compete for hydration and maintain solubility in the presence of high salt concentrations while retaining functionality (Karan et al. 2012). Non-halophilic proteins generally lose function under high salt conditions due to loss of hydration, denaturation, aggregation, and precipitation by salting-out (Danson and Hough 1997).

## Bioinformatic analysis

Genome-wide analysis of the first available haloarchaeal genome sequence for *Halobacterium* sp. NRC-1 led to the discovery of highly acidic, negatively charged proteins, with a unimodal isoelectric point distribution (pI 4.2), in contrast to the bimodal pI distributions seen for all other

organisms (DasSarma and DasSarma 2015; Kennedy et al. 2001). Amino acid composition analysis showed that there are nearly twice as many acidic glutamic and aspartic acid residues (~16%) in haloarchaeal proteins compared to basic arginine and lysine residues (~8%). Glutamate residues in particular are known to have superior water-binding activity, and constitute the larger fraction, almost 9%, in haloarchaeal proteins (Karan et al. 2012).

Modeling and structural analysis of haloarchaeal proteins confirmed that acidic residues at the surface play a significant role in binding of essential water molecules and salt ions, preventing protein aggregation and providing flexibility (Kennedy et al. 2001; Karan et al. 2012). Over 80% of glutamic acid residues are located on surface of proteins and this single characteristic likely contributes to the ability of haloarchaeal proteins to maintain their protein hydration. Conversely, reduction in the salt concentration can result in charge repulsion between negatively charged proteins, leading to unfolding and loss of function (Karan et al. 2012; Kennedy et al. 2001). Additional protein mutagenesis studies confirmed the importance of acidic residues, including clusters of aspartate and glutamate residues on the surface (Esclapez et al. 2007). Structural analysis showed the extended conformation of acidic residues pointed away from the protein surface, alternation in hydrophobic residues, and either burying of lysine side chains or their replacement by less hydrophilic arginine residues (Britton et al. 2006; Karan et al. 2012).

### Proteomic analysis

Haloarchaea have been analyzed for encoded proteins that may be key to their adaptation to extreme environments using best reciprocal hits analysis to identify those which are conserved in the clade (Capes et al. 2012) (Fig. 2). Among the nearly 800 conserved proteins, over 50 signature proteins were present in all the genomes included in the study and identified as potentially important to survival in the extreme environments in which Haloarchaea are found. While the function of most remains unknown, one of the *Halobacterium* sp. NRC-1 signature proteins, Ral, has been experimentally shown to function as a likely accessory protein in double-stranded DNA break repair important for desiccation and radiation tolerance (DeVeaux et al. 2007; Karan et al. 2014).

The proteome of the Antarctic haloarchaeon, *H. lacusprofundi*, has been extensively studied by comparative genomic analysis (DasSarma et al. 2013). After sequencing of the *H. lacusprofundi* genome, the proteome was found to be highly acidic, with an average pI of 4.6, and a high percentage (17%) of acidic residues resulting from an increase in aspartic and glutamic acid residues (Anderson et al. 2016). Amino acid composition of its predicted proteins was compared to

other Haloarchaea by reciprocal blasting to address cold-tolerance of the proteins (DasSarma et al. 2013). One *H. lacusprofundi* protein, a family 42  $\beta$ -galactosidase, which functions equally well in high concentrations of KCl and NaCl, was used as a model enzyme for studying the necessary adaptations to low temperatures at high salinity (Karan et al. 2013).

### Model polyextremophilic enzyme

The remarkable polyextremophilic characteristics of  $\beta$ -galactosidase enzyme from *H. lacusprofundi* were studied biochemically and by mutagenesis, after overexpression and purification from *Halobacterium* sp. NRC-1 (Karan et al. 2013). Its optimal activity was found to occur at a concentration of 4.5 M salts in either KCl or NaCl. It was also found to be active over a wide temperature range, from subzero temperatures where water remains liquid due to freezing point depression from the high salt concentrations, to temperatures as high as 70 °C, with an optimum of 50 °C (Karan et al. 2013). The ability to measure enzyme activity at subzero temperatures in nearly saturated salinity made the *H. lacusprofundi* enzyme unique among this family of  $\beta$ -galactosidases.

*H. lacusprofundi*  $\beta$ -galactosidase activity was also characterized by steady-state kinetics with various concentrations of  $\text{MgCl}_2$ ,  $\text{NaClO}_4$ , and  $\text{Mg}(\text{ClO}_4)_2$ , salts of considerable interest as a result of their detection on Mars (Hecht et al. 2009). Magnesium acted as a weak competitive inhibitor ( $K_i = 0.3$  M), likely binding to the active site and blocking access, while  $\text{NaClO}_4$  acted as an even weaker noncompetitive inhibitor ( $K_i = 2$  M), consistent with binding elsewhere on the protein and causing conformational changes (Laye and DasSarma 2018).  $\text{Mg}(\text{ClO}_4)_2$  acted as a stronger mixed inhibitor ( $K_i = 0.04$  M), exhibiting the combinatorial effects of both magnesium and perchlorate ions. Nevertheless, these studies and others aimed at growth inhibition showed that these Haloarchaea are able to tolerate concentrations of magnesium and perchlorate ions orders of magnitude higher than those expected on Mars (Laye and DasSarma 2018).

The *H. lacusprofundi*  $\beta$ -galactosidase enzyme was aligned to mesophilic and halophilic homologs to identify conserved residues that were diverged in the cold-active protein. In these related proteins of ~800 amino acid residues, only six residues were conserved in the mesophiles while being diverged in the cold-adapted species. These six residues were then mutated to determine whether they were responsible for conferring cold-adaptation to the protein (Laye et al. 2017). Based on steady-state kinetic analysis, all six residues played a measurable role in conferring cold-adaptation to the enzyme and mutating them affected  $K_m$ ,  $k_{cat}$ , or both, resulting in less cold-active enzymes. Molecular modeling showed small perturbations at the surface or in



the interior of the protein, at considerable distances from the active site, with effects on both binding and catalytic efficiency.

## Pigment biosynthesis

Most Haloarchaea such as *Halobacterium* spp. produce isoprenoid pigments that are important for their photobiology, functioning in photoprotection, photorepair, and phototrophic growth, and have the potential for astronomic detection (DasSarma et al. 2001; DasSarma and Schwieterman 2019). These pigments have been studied since the 1970s by purification and biochemical characterization using chromatographic and spectroscopic methods, which has led to the deciphering of many steps in their biosynthetic pathway (Fig. 3) (Kushwaha et al. 1976). Understanding of this pathway has been further advanced by isolation of mutants (both natural and constructed) and analysis of biochemical and phenotypic traits coupled with phenotypic, biochemical, genetic, and genomic analyses.

## Carotenoids and retinal

Haloarchaeal pigments include red–orange carotenoids, primarily C-50 bacterioruberins, characteristic of *Halobacterium* spp., C-40 lycopene and  $\beta$ -carotene, also common

in plants, and retinal, a C-20 vitamin A aldehyde similar to visual pigments in higher organisms (Armstrong et al. 1990; Kushwaha et al. 1976; Sumper et al. 1976). In the first step of the carotenoid pathway, two C-20 units are coupled to form the C-40 carotene phytoene by phytoene synthase. Phytoene is then converted to phytofluene,  $\beta$ -carotene, and lycopene by the action of one or more phytoene desaturases.

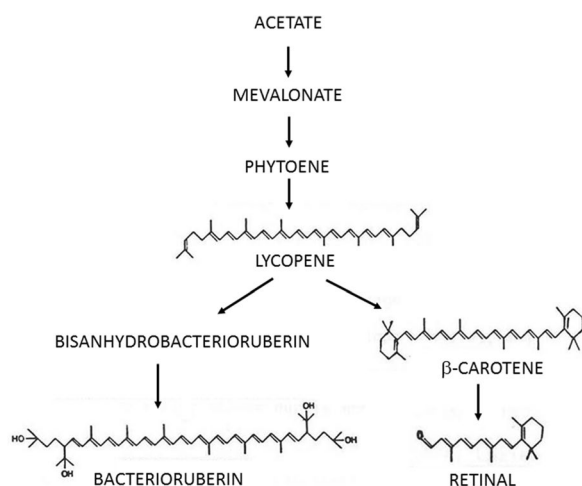
The carotenoid pathway diverges at lycopene, with one branch producing  $\beta$ -carotene through the action of lycopene cyclase followed by synthesis of retinal from oxidative cleavage of  $\beta$ -carotene by  $\beta$ -carotene monooxygenase (Fig. 3). The other branch produces longer C-50 bacterioruberins by the addition of two C-5 units to lycopene by the action of lycopene elongase, followed by hydroxylation and reduction by one or more bacterioruberin hydroxylases or desaturases (Yang et al. 2015). The bacterioruberins are the most abundant carotenoids produced in Haloarchaea and have been found to be involved in photoprotection and photorepair of DNA damage from UV radiation, while retinal is produced in sufficient quantities to serve as a chromophore for opsins used in phototrophy and phototaxis (DasSarma et al. 2001; McCready 1996; Stoeckenius and Bogomolni 1982).

Carotenoid biosynthesis has been characterized genetically in *Halobacterium* sp. NRC-1 and similar species. Studies established the conversion of lycopene to  $\beta$ -carotene followed by oxidative cleavage of  $\beta$ -carotene to retinal, as well as the elongation of lycopene to bisanhydrobacterioruberin and its hydration to a variety of bacterioruberins (Fang et al. 2010; Yang et al. 2015). Mutants lacking bacterioruberins have been reported, occurring spontaneously by insertions of ISH elements in genes coding lycopene elongase (Dummer et al. 2011).

## Purple membrane

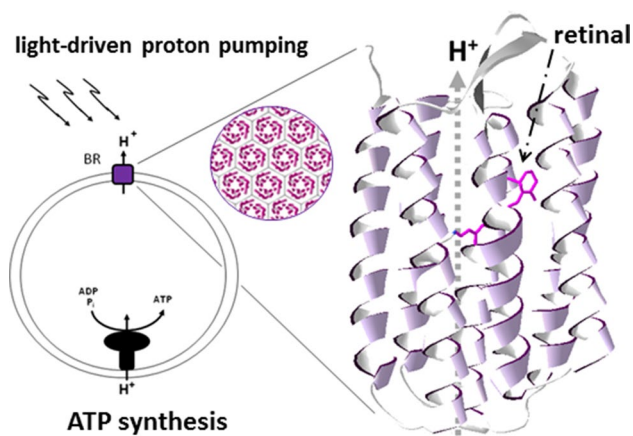
Haloarchaea contain retinal proteins that bind covalently in a 1:1 ratio via a Schiff's base linkage to lysine (Bayley et al. 1981). The most abundant retinal protein is bacteriorhodopsin (Fig. 4), a light-driven proton pump that forms a two-dimensional crystalline lattice of trimers in the purple membrane, a specialized region of the cell membrane (Stoeckenius and Bogomolni 1982). Depending on the abundance of purple membrane in cells and the light intensity in the environment, a period of phototrophic growth may be supported (Sumper et al. 1976). The purple membrane can be isolated by sucrose gradient centrifugation, and has been extensively studied for its functional characteristics (Krebs and Khorana 1993). The genes for purple membrane synthesis have been studied by transcriptomic analysis and isolation of mutants of the opsin or chromophore (DasSarma et al. 2012).

Mutants in the pathway include orange-colored variants that were the result of defects in purple membrane synthesis



## Photoprotection and repair Phototrophic growth

**Fig. 3** Pigment biosynthesis in Haloarchaea. Biosynthesis of common pigments in Haloarchaea such as *Halobacterium* species proceeds from acetate via mevalonate to lycopene where the pathway splits to form the bacterioruberins or  $\beta$ -carotene and retinal. Bacterioruberins have been implicated in photoprotection and repair while retinal acts as the chromophore in purple membrane used for phototrophic growth (DasSarma et al. 2001)



**Fig. 4** Phototrophic capability of Haloarchaea. Haloarchaea such as *Halobacterium* sp. NRC-1 contain the retinal protein bacteriorhodopsin (BR) which is an outwardly directed light-driven proton pump producing a proton-motive gradient that drives ATP synthesis using ADP and  $P_i$  (left). Trimers of BR form a hexagonal lattice in the membrane (circular inset, center). BR's seven transmembrane  $\beta$ -helical segments are shown in ribbon form (right) with the bound retinal (stick structure) involved in promoting transport of protons ( $H^+$  ions) across the membrane (DasSarma and Schwieterman 2019)

as well as those which were deep purple from over-production of purple membrane (DasSarma 1989). The bacteriorhodopsin (*bop*) gene was shown to be frequently subject to insertion by ISH elements and led to the discovery and analysis of additional upstream genes in the regulon (Yang et al. 1996; Baliga et al. 2001). They include *bat*, a regulatory gene, deletion of which resulted in elimination of purple membrane production without inhibiting the synthesis of bacterioruberins (DasSarma et al. 2012).

Mutants defective in retinal synthesis were also isolated and studied. Deletions in two genes, *brp* and *blh*, coding for  $\beta$ -carotene monooxygenases, which cleave  $\beta$ -carotene into two molecules of retinal, blocks the final step in retinal synthesis (Peck et al. 2001). Deletion of *brp* resulted in decreased bacteriorhodopsin and retinal levels, while bacteriorhodopsin levels were normal, and  $\beta$ -carotene levels were increased, while deletion of *blh* resulted in reduced bacteriorhodopsin accumulation. In a double-deletion mutant ( $\Delta brp \Delta blh$ ) there was no detectable level of bacteriorhodopsin or retinal, without changes in bacteriorhodopsin accumulation while  $\beta$ -carotene levels were increased. The two genes, *brp* and *blh*, are also likely differentially regulated, with the *brp* gene located directly upstream of the *bop* gene and the *blh* gene located in a gene cluster with  $\beta$ -carotene cyclase and subunits of ATP synthase (DasSarma et al. 2012; Peck et al. 2001). Finally, the cytochrome P450 monooxygenase was also implicated in carotenoid biosynthesis, deletion of which increased bacterioruberin while decreasing purple membrane content (Müller et al. 2019).

## Astronomical biosignatures

Surface features indicative of living biomass, including haloarchaeal pigments, have been proposed as potential astronomical biosignatures (Schwieterman et al. 2018; Seager et al. 2005). On the modern Earth, chlorophyll pigments produce a reflective spectroscopic edge, i.e., the “red edge” at  $\sim 700$  nm, where vegetation transitions from primarily absorbing visible light to reflecting and scattering near-infrared light (Gates et al. 1965; Knipling 1970). A wide variety of non-photosynthetic organisms, including phototrophic microorganisms, have pigments that also produce “edge” wavelengths in reflectance spectra and could conceivably be remote signatures if the biomass is sufficiently well-distributed over a planetary surface (Hegde et al. 2015; Lingam and Loeb 2017; Schwieterman et al. 2015).

Among potential astronomical biosignatures, haloarchaeal pigments, which may have predated photosynthetic pigments, are attractive candidates (Schwieterman et al. 2018; DasSarma and Schwieterman 2019). Brine lakes with pigmented blooms of diverse Haloarchaea are widely distributed and represent potential analogs for remote life detection on other planets. One of the largest such lakes, Great Salt Lake in the United States Great Basin, extends over ca. 4000 square kilometers, with blooms of halophilic microorganisms observed from space (DasSarma et al. 2016). In culture, *Halobacterium* sp. NRC-1 exhibits pink floating biomass due to cell buoyancy and refraction of light by hollow gas vesicle nanoparticle organelles as well as high quantities of carotenoid pigments and purple membrane (DasSarma et al. 2006; DasSarma 2007). *H. lacusprofundi* cultures produce even higher levels of carotenoid pigments than *Halobacterium* sp. NRC-1, and appear bright orange.

Haloarchaeal pigments have been remotely detected in the hypersaline solar salterns of South San Francisco Bay used for mining sea salt. These blooms are readily observed from airplanes and can take on different hues, including orange, red, or purple, at different times. The Jet Propulsion Lab generated 10-year record of remote spectral information using an airborne visible and infrared imaging spectrophotometer (Dalton et al. 2009). The strongest reflection spectral signals were seen in the most saline ponds ( $> 200$  g/L), where carotenoid and bacteriorhodopsin peaks (reflectance troughs) were observed at 500–570 nm. At lower salinities ( $< 50$  g/L), chlorophyll absorption spectra were most readily visible.

## Future studies

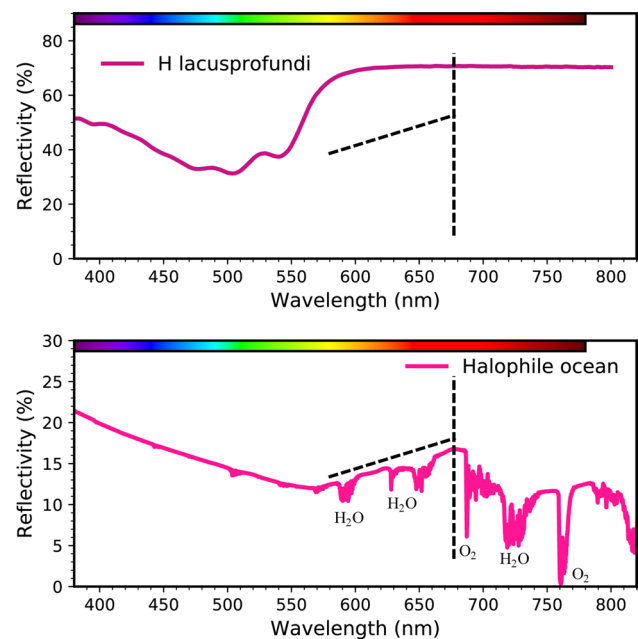
The science of exoplanets is rapidly moving from detection and bulk characterization to atmospheric spectroscopy

and studies of chemical make-up. While current technology limits the study of Earth-size planets in the habitable zones of nearby stars, future-space-based telescope concepts such as LUVOIR or HabEx would have the capacity to directly image these worlds and characterize their atmospheres and surfaces at visible wavelengths (Bolcar et al. 2017; Roberge and Moustakas 2018). To better inform the design and implementation of these missions, astrobiologists have worked to catalog the ensemble of potentially observable biosignatures—remote spectral or photometric indicators of life (Schwieterman et al. 2018).

Haloarchaea and other retinal-based phototrophs (DasSarma and DasSarma 2017; Finkel et al. 2013) produce a spectral signature different than, but complementary to vegetation. In contrast to photosynthetic vegetation and their 700-nm red-edge, laboratory studies of *H. lacusprofundi* produce a ~550 nm “green-edge” (Fig. 5) (DasSarma and Schwieterman 2019). Modeling suggests that blooms of such Haloarchaea suspended in an evaporite solution, including radiative effects from the scattering and absorption of water, combined with the increasing microbial reflectivity at green, yellow, and red wavelengths, would produce a peak reflection at ~680 nm (Fig. 5). This wavelength is relatively transparent to atmospheric absorption and scattering. Given sufficient surface coverage, this pigment signature may be detectable in the overall planetary spectrum of an exoplanet (Schwieterman et al. 2015).

The use of Haloarchaea and other retinal phototrophs for a remote astronomical biosignature is specially promising because these microorganisms can acquire light energy for metabolism and are known empirically to produce macroscopic and remotely observable blooms on Earth (Dalton et al. 2009; DasSarma 2006, 2007; DasSarma et al. 2016). Moreover, the Purple Earth hypothesis suggests that planets may evolve through a period when these pigments may dominate and therefore may represent an early stage of evolution (DasSarma and Schwieterman 2019). If the prevailing conditions on an exoplanet are similar, it is possible that Haloarchaea-like signatures may be remotely observable.

Fortunately, visible wavelengths are likely to be included in future space and ground-based observatories capable of imaging planets in their habitable zones (Fujii et al. 2018), and so should detect sufficiently strong “edge” biosignatures. It should be noted, however, that the signal-to-noise requirements may be more challenging compared to, for example, detecting molecular oxygen in the atmosphere (Brandt and Spiegel 2014). Future measurements are needed to thoroughly survey the reflectance spectra of Haloarchaea and other retinal phototrophs in vivo and in solution and enumerate potential false positives from abiotic surfaces, such as minerals (e.g., Clark et al. 2007). Additional modeling efforts are also essential to ascertain the minimum surface coverage and cell density required to detect these surface



**Fig. 5** Reflective green edge from haloarchaeal pigments. A laboratory reflection spectrum of *H. lacusprofundi* (top panel) and an environmentally characterized halophile bloom under a simulated Earth atmosphere (bottom panel), including atmospheric absorption and scattering, are shown, plotting wavelength in nanometers (nm) (abscissa) versus percent reflectivity (ordinate) (DasSarma and Schwieterman 2019). Dashed lines are shown for reference and strong water (H<sub>2</sub>O) and oxygen (O<sub>2</sub>) interference bands labeled

biosignatures on nearby exoplanets, given the likely capabilities of future space telescopes.

## Concluding remarks

Haloarchaea represent outstanding extremophiles for astrobiology studies since they survive in a variety of planetary environments and are relevant for in situ life detection. Their evolutionary, physiological, and spectroscopic properties make them of interest when considering the origin of life, biochemical and metabolic capabilities, and pigments that may serve as potential remote biosignatures. Future laboratory and modeling studies of these extremophiles together with large space-based telescopes that are planned for future astronomical observations are likely to lead to new avenues of highly consequential research, with the potential for answering questions about the rarity or ubiquity of life in the universe.

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