International Journal of Astrobiology 16 (4): 321–327 (2017)
doi:10.1017/S1473550416000410

Survival of halophilic Archaea in Earth's cold stratosphere

P. DasSarma[1], V.J. Laye[1], J. Harvey[2], C. Reid[2], J. Shultz[2], A. Yarborough[2], A. Lamb[2], A. Koske-Phillips[2], A. Herbst[2], F. Molina[2], O. Grah[2], T. Phillips[2] and S. DasSarma[1]

[1]*Department of Microbiology and Immunology, Institute of Marine and Environmental Technology, University of Maryland School of Medicine, Baltimore, MD 21202, USA e-mail: pdassarma@som.umaryland.edu*
[2]*Earth to Sky Calculus and Spaceweather.com, Bishop, CA, USA*

Abstract: Halophilic Archaea are known to tolerate multiple extreme conditions on Earth and have been proposed as models for astrobiology. In order to assess the importance of cold-adaptation of these microorganisms in surviving stratospheric conditions, we launched live, liquid cultures of two species, the mesophilic model *Halobacterium* sp. NRC-1 and the cold-adapted Antarctic isolate *Halorubrum lacusprofundi* ATCC 49239, on helium balloons. After return to Earth, the cold-adapted species showed nearly complete survival while the mesophilic species exhibited slightly reduced viability. Parallel studies found that the cold-adapted species was also better able to survive freezing and thawing in the laboratory. Genome-wide transcriptomic analysis was used to compare the two haloarchaea at optimum growth temperatures versus low temperatures supporting growth. The cold-adapted species displayed perturbation of a majority of genes upon cold temperature exposure, divided evenly between up-regulated and down-regulated genes, while the mesophile exhibited perturbation of only a fifth of its genes, with nearly two-thirds being down-regulated. These results underscore the importance of genetic responses of *H. lacusprofundi* to cold temperature for enhanced survival in the stratosphere.

Received 22 August 2016, accepted 15 September 2016, first published online 27 October 2016

Key words: cold temperature, extremophiles, *Halobacterium* sp. NRC-1, *Halorubrum lacusprofundi*, helium balloon, Mars analogue, stratosphere.

Introduction

The recent discovery of brine flows on the surface of Mars has intensified interest in the extremophilic character of extremely halophilic microorganisms in relation to astrobiology (Mancinelli *et al.* 2004; DasSarma 2006; Horneck *et al.* 2010; Leuko *et al.* 2014). Flowing brines were first noted as seasonal dark streaks or recurring slope lineae (RSL) on the walls of Garni crater observed from images captured by NASA's Mars Reconnaissance Orbiter (McEwen *et al.* 2011). The occurrence of RSLs at subzero temperatures indicated that frozen brines melt and flow seasonally on the Martian surface (Ojha *et al.* 2015). This prediction was also supported by spectroscopic evidence for hydrated sodium and magnesium chloride, chlorate and perchlorate salts at the Phoenix lander site (Kounaves *et al.* 2014; Toner *et al.* 2014). Interest in Mars Special Regions, such as the Phoenix site, is heightened by the existence of usable metabolic energy and its potential for enabling habitability (Stoker *et al.* 2010; Schuerger *et al.* 2013; Rummel *et al.* 2014).

On Earth, hypersaline brines are nearly ubiquitous and generally thalassic, with high biological productivity promoted by sunlight driving pigment production and a variety of primary metabolic processes. A diversity of halophilic microorganisms flourish, originating from all three branches of life: Archaea, Bacteria and Eukarya (DasSarma & DasSarma 2012). Halophilic Archaea (Haloarchaea) are able to tolerate high salinity primarily through the salt-in strategy, producing negatively charged proteins that remain soluble and compete successfully with ions for hydration (Karan *et al.* 2012; DasSarma & DasSarma 2015, see also Goh *et al.* 2011). Bacteria usually survive by the salt-out strategy, synthesizing or taking-up zwitterionic compatible solutes, which helps maintain osmotic balance with external salts, while Eukarya generally produce neutral polyols, e.g. glycerol, for the same purpose (DasSarma & DasSarma 2012). Combinations of these mechanisms operate in some species (DasSarma & DasSarma 2015).

Among the Haloarchaea, *Halobacterium* sp. NRC-1 has been extensively studied for its extremophilic character (DasSarma *et al.* 2006; DasSarma & DasSarma 2012). This species is capable of tolerating high concentrations of sodium and potassium chlorides and perchlorates (Coker *et al.* 2007; DasSarma *et al.* unpublished). In addition to high salinity, this strain is slightly thermotolerant with optimum growth at 42°C and survival at 49–50°C (Coker *et al.* 2007) and is highly resistant to ultraviolet (UV) and ionizing radiation (McCready *et al.* 2005; DeVeaux *et al.* 2007). Genetic, genomic and transcriptomic studies have established a wide range of survival mechanisms operating in *Halobacterium,* including presence of highly acidic proteins and direct photorepair, double-stranded gap repair and nucleotide excision repair systems (Crowley *et al.* 2006; Boubriak *et al.* 2008; Karan *et al.* 2014).

Another haloarchaeal strain relevant to astrobiology, *Halorubrum lacusprofundi*, was isolated from Deep Lake in the Vestfold Hills of Antarctica (Franzmann *et al.* 1988). Deep Lake is perennially cold, with the temperature remaining subzero for more than 6 months of the year. However, Deep Lake does not freeze, even when temperatures drop to −18°C due to freezing-point depression from high (28%) salinity. *H. lacusprofundi* is capable of growth down to −2°C and is well-adapted to this environment (Reid *et al.* 2006). *H. lacusprofundi* biofilms have been reported at the lowest growth temperatures and may serve to enhance survival. The *H. lacusprofundi* genome has been completely sequenced and its proteins exhibit reduced surface acidity and internal amino acid substitutions characteristic of enhanced internal flexibility (Anderson *et al.* 2016; DasSarma *et al.* 2013; Karan *et al.* 2013).

In order to evaluate the astrobiological potential of Haloarchaea, we sought to compare the ability of *Halobacterium* sp. NRC-1 and *H. lacusprofundi* to survive trips into the stratosphere. While Earth's stratosphere exhibits multiple extremes not dissimilar to those found on the surface of Mars (Smith *et al.* 2011; Smith 2013), including cold temperatures, high radiation fluxes and low pressures, our primary interest in this study was to assess the importance of tolerance to extremely cold temperatures in survival. Our results show that while both halophiles are able to survive trips into the stratosphere, *H. lacusprofundi* exhibits greater viability and a more robust transcriptomic response in the extremely cold temperatures experienced.

Materials and methods

Helium balloon launching and recovery

Natural rubber 1.6 kg balloons filled with 5.66 m^3 of helium were used to provide the free lift required to pull a 2.2 kg capsule to the stratosphere at a rate of approximately 300 m min^{-1}. Helium balloons were used for high-altitude launches from the Sierra Nevada Mountains of central California between February and July 2015. Items mounted on the outside of the payload included a cryogenic temperature probe, GPS 'SPOT' trackers and a GPS altimeter. Tubes containing Haloarchaea were affixed to the exterior of the payload in order to fully expose them to environmental conditions from ground level to altitudes of 33–36 km above sea level. When balloons reached peak altitude, they exploded and the payload plummeted towards Earth. A parachute slowed the descent, resulting in a soft landing. Each payload was recovered within about an hour of landing, by following the SPOT tracker GPS coordinates to the landing site.

Haloarchaeal culturing and laboratory analysis

Halobacterium sp. NRC-1 was cultured in CM^+ medium at 42°C while *H. lacusprofundi* ATCC 49239 was cultured in ATCC1682 Deep Lake medium at 30°C, as previously described (Berquist *et al.* 2006; Reid *et al.* 2006; Anderson *et al.* 2016). Cultures were grown to late-log phase and aliquoted into cryovials for shipments by courier from Maryland to California and launching into the stratosphere (Fig. 1). To determine colony forming units, cultures were diluted and plated on agar plates and colonies counted after 10 days incubation at the optimal growth temperatures. In each case, experiments were done in triplicate and standard deviations calculated using Microsoft Excel. Cells were photographed in a Nikon Labophot phase contrast microscope using a Nikon D90 DSLR. Pigment analysis was conducted after pelleting cells by centrifugation (10 min, 10 000 × ***g***), hypotonic lysis in deionized water and spectroscopy using a Shimadzu UV-1601 spectrophotometer. For viability assays after freeze-thaw, cultures in cryotubes were placed in a −70°C freezer for 2 h, followed by thawing at room temperature and determining colony forming units, as described above.

Genome-wide transcriptomic analyses

Custom whole genome DNA oligonucleotide microarrays for *Halobacterium* sp. NRC-1 and *H. lacusprofundi* were manufactured by Agilent Corporation (Coker *et al.* 2007). To compare transcriptional profiles, triplicate cultures of *Halobacterium* sp. NRC-1 grown at 42 or 15°C and *H. lacusprofundi* grown at 30 or 4°C, both to an OD_{600} of 0.3. The temperatures reflected either optimal or minimal growth temperatures for transcriptomic analysis. After collecting cells by centrifugation, RNA was isolated and pooled, Cy3 and Cy5-labelled cDNA was synthesized and labelled cDNA was hybridized to duplicate microarrays, washed and scanned on an Agilent DNA microarray scanner (model no. G2565BA), as previously described (Müller & DasSarma 2005; Coker *et al.* 2007). Agilent Feature Extraction software was used for image analysis and processing of the microarray image file. Statistical analyses were performed as previously described using a 1.5-fold change threshold cutoff. Expression data were aligned with protein families (conserved Haloarchaeal orthologous genes (cHOGs)) using MS Excel (DasSarma *et al.* 2010; Capes *et al.* 2012).

Results

Flights into the stratosphere

Halobacterium sp. NRC-1 and *H. lacusprofundi* grown in liquid cultures in the laboratory in Maryland were aliquoted and shipped to California by overnight courier (Fig. 1). Experimental samples were launched to the stratosphere affixed to the payloads of Earth to Sky Calculus high-altitude helium balloons while control aliquots were either stored on the ground in Maryland or shipped to and returned from California without balloon flights. Experimental vials were launched to heights of 36 km, with flight times of 2.5 h from launch to landing, including 1 h in the stratosphere. The minimum temperature recorded was −65°C, with UV-C doses of 18 J m^{-2} and air pressure as low as 230 Pa (Jacob 1999). Samples were recovered typically within an hour and shipped by overnight courier to Maryland for analysis.

Experimental and control samples of *Halobacterium* sp. NRC-1 and *H. lacusprofundi* were analysed visually,

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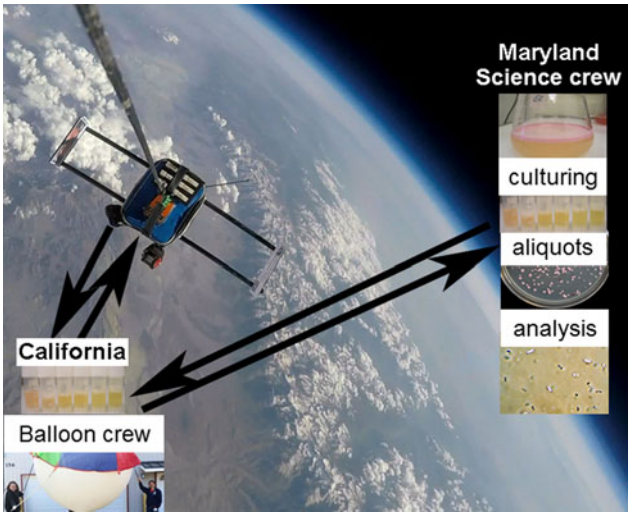

Fig. 1. Experimental coordination of balloon launches and Haloarchaeal microbiology. Cultures of *Halobacterium* sp. NRC-1 and *H. lacusprofundi* were grown in the University of Maryland and sent by courier to Earth to Sky Calculus for launching into the stratosphere. Samples were returned to Maryland for analysis.

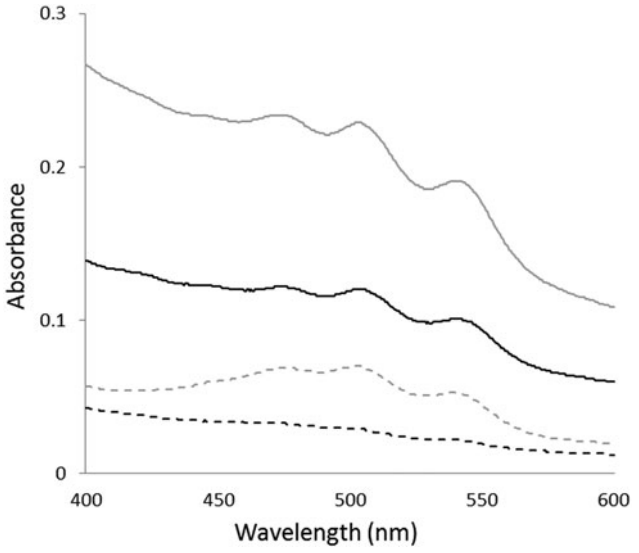

Fig. 2. Visible spectra of *Halobacterium* sp. NRC-1 (dashed) and *H. lacusprofundi* (solid) lysates, either balloon flown (black) or shipped by courier (gray).

spectrophotometrically and microscopically. Balloon-flown samples were found to be partially cleared and bleached upon visual inspection compared with the courier flown and ground-bound control samples. Examination of cells by phase contrast microscopy indicated that *Halobacterium* sp. NRC-1 cells had lost their refractive gas vesicles (data not shown), which normally provide buoyancy and promote cell flotation for phototrophic growth in their hypersaline environment (DasSarma *et al*. 2012). Morphological differences were less pronounced for *H. lacusprofundi* cells, which naturally lack gas vesicles and exhibit a higher level of pigmentation. The partial loss of pigmentation for both organisms after flights to the stratosphere was confirmed by UV-VIS spectroscopy, with reduced absorption detected at wavelengths characteristic of bacterioruberins (Fig. 2).

Survival difference after stratospheric flights corresponds to freeze-thaw tolerance

In order to determine the survival of *Halobacterium* sp. NRC-1 and *H. lacusprofundi*, dilutions of cultures remaining in Maryland and cultures that were shipped to and from California with or without experiencing the stratosphere from launches on balloons, were plated in triplicate. Cultures with or without shipping showed no statistical difference in survival, indicating that there was no significant effect on viability of transit by courier (Fig. 3). Interestingly, cultures of both *Halobacterium* sp. NRC-1 and *H. lacusprofundi* flown to the stratosphere appeared to show a significant degree of survival, although the Antarctic species showed relatively better survival than the mesophile (Fig. 3). Quantitative analysis indicated 50% survival for *H. lacusprofundi* and 5% for *Halobacterium* sp. NRC-1.

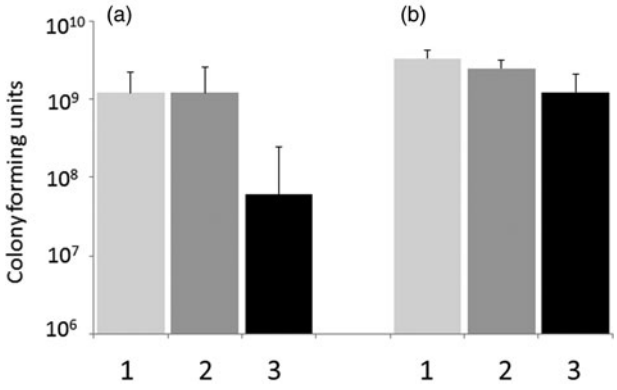

Fig. 3. Survival analysis of *Halobacterium* sp. NRC-1 (A) and *H. lacusprofundi* (B). Colony forming units are indicated on the *y*-axis for cultures remaining on the ground (1), courier transported (2) and exposed to the stratosphere (3). Error bars indicate standard deviations of three measurements.

In order to determine whether freeze-thaw tolerance was responsible for the observed difference in survival, we froze both *Halobacterium* sp. NRC-1 and *H. lacusprofundi* cultures in the laboratory. After thawing, survival of each Haloarchaeaon was quantified by serial dilution and plating. *H. lacusprofundi* was found to be considerably more tolerant to freeze-thaw than *Halobacterium* sp. NRC-1 (88 versus 16% survival, respectively) (Fig. 4). These findings showed that the better freeze-thaw survival of the cold-adapted Antarctic isolate, *H. lacusprofundi*, compared with the mesophilic *Halobacterium* sp. NRC-1, parallels the observed higher survival in the stratosphere (Fig. 3). Interestingly, freeze-thaw also led to partial loss of pigmentation in both Haloarchaea and loss of intracellular gas vesicles in *Halobacterium* sp. NRC-1 (data not shown).

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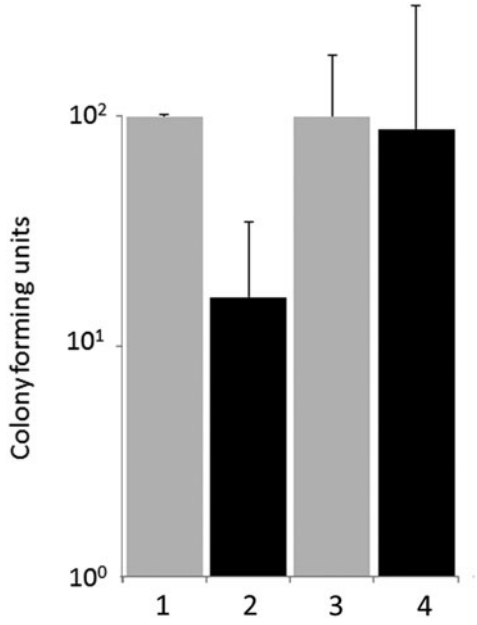

Fig. 4. Survival analysis of *Halobacterium* sp. NRC-1 and *H. lacusprofundi* exposed to freeze-thaw in the laboratory. Ambient temperature (gray) versus freeze-thawed (black) samples. *Halobacterium* sp. NRC-1 (1 and 2) and *H. lacusprofundi* (3 and 4). Error bars indicate standard deviations of three measurements.

Genome-wide transcriptomic analysis

In order to address the difference in cold-survival of *Halobacterium* sp. NRC-1 and *H. lacusprofundi* at the transcriptomic level, we compared genome-wide transcriptomes of the two microorganisms at low, versus optimum growth temperatures using DNA microarrays. For *Halobacterium* sp. NRC-1, DNA microarrays comparing the optimal temperature of 42°C to the low temperature of 15°C had been conducted earlier (Coker *et al.* 2007). We compared these data to transcriptomic data for *H. lacusprofundi* near its optimal growth temperature of 30°C and low growth temperature of 4°C. The numbers of genes with significantly changed transcription levels are shown in Table 1.

The findings showed that gene expression in *H. lacusprofundi* is much more responsive to temperature differences than in *Halobacterium* sp. NRC-1 (Table 1). Nearly 60% of *H. lacusprofundi* genes were up- or down-regulated by ≥1.5-fold in this cold-adapted haloarchaeon with nearly equal numbers of genes changing in each direction, 31% up-regulated and 29% down-regulated. By contrast, <20% of genes in the mesophile *Halobacterium* sp. NRC-1 were regulated to the same extent, with twice as many down-regulated as up-regulated genes. Down-regulated genes are likely primarily growth rate-dependent since colder temperatures slow growth (Coker *et al.* 2007).

Regulated genes in the two halophiles were determined for conserved genes using the cHOGs database (DasSarma *et al.* 2010; Capes *et al.* 2011, 2012). The results for the subset of cHOGs were very similar to the genome as a whole, with 55% of *H. lacusprofundi* compared with 20% of *Halobacterium* sp. NRC-1 orthologs significantly changed (Table 1). Most of the up-regulated cHOGs in *Halobacterium* sp. NRC-1 were also similarly perturbed in *H. lacusprofundi* (29 out of 43). Interestingly, some of the most highly up-regulated genes were conserved transcriptional regulators, general transcription factors and chaperones (Table 2). For transcription factors and regulators, there were a larger number of genes up-regulated in *H. lacusprofundi* compared with *Halobacterium* sp. NRC-1, while the number was nearly the same for chaperones. Orthologs of nearly all of the perturbed *Halobacterium* sp. NRC-1 genes were also found to be changed in *H. lacusprofundi*. These findings are consistent with a common core response to cold-temperature, as reflected by changes observed in both Haloarchaea, with an additional strong and diverse response to cold and freezing conditions specifically in the Antarctic *H. lacusprofundi*.

Discussion

Halophilic Archaea have been proposed to be excellent candidates for astrobiology (DasSarma 2006). We tested effects of stratospheric conditions on survival of live, metabolically active Haloarchaea by launching two species on helium balloons. Our results showed that both species, *Halobacterium* sp. NRC-1 and *H. lacusprofundi*, can survive stratospheric flights, with the latter cold-adapted Antarctic species displaying superior survival ability compared with the former mesophilic species. *H. lacusprofundi* therefore appears better suited for stratospheric conditions.

Enhanced survival of *H. lacusprofundi* over *Halobacterium* sp. NRC-1 in the stratosphere is primarily due to its better adaptation to cold temperatures (Fig. 4). The stratosphere is very cold, between −10 and −65°C (Jacob 1999), with the lower temperature resulting in freezing of the hypersaline culture media. Upon return to the troposphere or after landing, Haloarchaeal cultures thawed. Since *H. lacusprofundi* experiences frigid subzero temperatures for 8 months of the year in its natural environment of Deep Lake in the Vestfold Hills of Antarctica, it is better capable of tolerating the cold freeze occurring in the stratosphere (Franzmann *et al.* 1988; Reid *et al.* 2006). In contrast, the mesophilic species isolated from a temperate climate does not appear to be able to withstand cold temperatures well and loses considerably more viability from the journey into the stratosphere (Coker *et al.* 2007).

The cold-adaptive mechanisms operating in *H. lacusprofundi* and *Halobacterium* sp. NRC-1 were found to have similarities and differences using genome-wide transcriptomic analysis (Tables 1 and 2) (Coker *et al.* 2007). *H. lacusprofundi* exhibited significantly higher percentage and numbers of cold-induced genes, including general transcription factors and transcriptional regulators. In *Halobacterium* sp. NRC-1, our prior work had shown that three transcription factors (TbpD, TfbA and TfbG) were involved in, or were responsive to cold temperatures, with only a single one, TfbG, displaying expression level perturbation by cold temperature (Coker & DasSarma 2007; Coker *et al.* 2007). *H. lacusprofundi* showed five transcription factors with gene expression responsive to

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Table 1. *Genes perturbed by low temperatures in* Halobacterium *sp. NRC-1 and* H. lacusprofundi

	Total no. of genes	Up-regulated (%)	Down-regulated (%)	Total up- or down-regulated (%)
Halobacterium sp. NRC-1 (42 versus 15°C)	2484	168 (6.76)	310 (12.48)	478 (19.24)
H. lacusprofundi (30 versus 4°C)	3562	1113 (31.16)	1024 (28.75)	2137 (59.99)
Halobacterium sp. NRC-1 genes in cHOGs	841	43 (5.11)	127 (15.10)	170 (20.21)
H. lacusprofundi genes in cHOGs	871	247 (28.36)	233 (26.75)	480 (55.11)
Number of cHOGs in both haloarchaea	784	29 (3.70)	27 (3.44)	56 (7.14)

Table 2. *Selected cold up-regulated genes in* Halobacterium sp. *NRC-1 and* H. lacusprofundi

Gene type and annotation		*Halobacterium* sp. NRC-1			*H. lacusprofundi*		
	HOG	Gene ID	Change	*P*-value	Gene ID	Change	*P*-value
Transcription factors							
TBP TATA-box binding family protein					Hlac_2818	2.91	0.01
TBP TATA-box binding family protein	44				Hlac_3413	2.09	0.48
TFB transcription initiation factor IIB	18	*tfb*G	2.26	0.26	Hlac_1495	3.60	0.00
TFB transcription initiation factor IIB	4				Hlac_1513	6.27	0.00
TFB transcription initiation factor IIB	4				Hlac_601	2.05	0.00
Transcriptional regulators							
Metal dependent repressor, DtxR family	624	*sir*R	3.75	0.21	Hlac_657	2.08	0.31
Transcriptional regulator, ArsR family	325	*boa*2	1.55	0.12	Hlac_1852	2.50	0.04
Transcriptional regulator, PadR family	1	vng751	1.80	0.00	Hlac_3648	11.60	0.13
Transcriptional regulator, PadR family	1				Hlac_3619	2.22	0.71
Transcriptional regulator, ArsR family	94				Hlac_395	2.93	0.00
Transcriptional regulator, ArsR family	731				Hlac_184	177.04	0.00
Transcriptional regulator, AsnC family	731				Hlac_3617	2.12	0.78
Transcriptional regulator, AsnC family	425				Hlac_2326	2.47	0.43
Transcriptional regulator, CopG family	109				Hlac_2067	3.57	0.00
Transcriptional regulator, CopG family	977				Hlac_524	3.35	0.00
Transcriptional regulator, TrmB family	43				Hlac_2144	2.10	0.13
Transcriptional regulator, XRE family	632				Hlac_273	3.99	0.05
Stress proteins and chaperones							
Cold-shock protein	2	*csp*D2	3.11	0.02	Hlac_1630	4.80	0.00
Cold-shock protein	2	*csp*D1	2.31	0.44	Hlac_1312	2.16	0.00
Small heat shock protein (HSP20 family)	237	vng283	1.81	0.07	Hlac_637	1.95	0.00
Thermosome, GroEL (HSP60 family)	320	*cct*B	1.79	0.01	Hlac_416	2.74	0.00
Prefoldin, alpha subunit	428	vng2465	1.68	0.00	Hlac_822	3.10	0.00
Prefoldin, beta subunit	403				Hlac_567	15.21	0.00
20S proteasome A and B subunits	127				Hlac_608	2.06	0.01
Predicted ATP-dependent protease	200	*lon*	1.48	0.25			

cold temperatures, two Tbps and three Tfbs. Similarly, *H. lacusprofundi* also exhibited a larger number of transcriptional regulators responding to cold temperatures, twelve versus only three in *Halobacterium* sp. NRC-1. For stress protein and chaperone genes, five orthologous genes were up-regulated in both, with only three non-orthologs varying between the two (two in *H. lacusprofundi* and one in *Halobacterium* sp. NRC-1). Together, these findings showed that while the core cold-stress response is quite similar in both Haloarchaea, the Antarctic halophile has a much larger and diverse set of cold-responsive genes compared with the mesophile. These findings likely account for *H. lacusprofundi*'s better survival under cold and freezing temperatures encountered in the stratosphere.

This is the first study of survival of live Haloarchaeal cultures under stratospheric conditions. Prior studies have examined the survival ability of a related species, *Halorubrum chaoviatoris*, from a Mexican intertidal marine area during a flight aboard ESA's Biopan 1 facility (Mancinelli *et al.* 1998). This experiment exposed desiccated cells to deep space conditions with considerably higher dose of radiation, leading to lowering of viability by 10^6-fold. Clearly, exposure of desiccated cells to space conditions is significantly more challenging for the microbes. The current study is more likely to reflect potential survival of Haloarchaea under low temperatures, in surface or subsurface brines, than unprotected exposure to the surface of Mars or deep space. Additional studies are needed to determine the combinatorial effects of several stressors, including cold temperature, UV-C and cosmic radiation and low pressures.

A variety of other microorganisms have been tested for survival under space and simulated Mars conditions and in the stratosphere. In one study of nearly two-dozen bacterial species exposed to cold, low pressure and anoxic conditions in the

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laboratory, only a single microbe, *Serratia liquefaciens* was found to survive all of the extremes (Schuerger *et al*. 2013). In this study, two extremophiles, *Deinococcus radiodurans* and *Psychrobacter cryohalolentis*, were not capable of survival under these conditions. Other studies have focused on spore forming bacteria, such as *Bacillus subtilis* (Nicholson *et al.* 2012; Wassmann *et al.* 2012) and *B. pumilus* SAFR-032. The quiescent spore forms of *B. pumilus* are highly resistant to UV radiation and cold temperatures (Calcott & MacLeod 1975; Vaishampayan *et al.* 2012).

Recently, several commercial bacterial cultures (*Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium*) and human-associated bacteria (*Proteus mirabilis* and *Pseudomonas aeruginosa*) were launched into the stratosphere on helium balloons (Chudobova *et al*. 2015). The survival of these bacteria was found to be significantly poorer than for the Haloarchaea in our study, especially *H. lacusprofundi*. Moreover, this study reported survival to be lower in the stratosphere than for a similar dose exposure to UV-C in the laboratory. However, the effects of temperature were not investigated in this study.

While the focus in our study has been survival in cold temperatures, we have also examined the effect of UV-C on the two Haloarchaea in the past (McCready *et al*. 2005; Crowley *et al.* 2006; Boubriak *et al.* 2008; our unpublished data). Our findings have shown that *Halobacterium* sp. NRC-1 and *H. lacusprofundi* are both quite tolerant to UV-C, with an LD_{50} of ~ 50 J m^{-2}. Additional experiments are needed to determine the relative importance of UV-C, low pressure and other stressors in the partial loss of viability of Haloarchaea in the stratosphere. However, such studies would require longer flights.

Conclusions

Halobacterium sp. NRC-1 and *H. lacusprofundi* are able to withstand exposure to stratospheric conditions. *H. lacusprofundi*, an isolate from a perennially cold, hypersaline lake in Antarctica retains greater viability, at least for the time periods experienced using helium balloons, a property likely resulting from better cold and freeze adaptation. Most of the genes in the genome of the Antarctic species are significantly regulated at cold temperatures, which likely results in its better adaptation to the cold conditions in its natural environment and in the stratosphere.

Acknowledgements

Our work was supported by National Aeronautics and Space Administration grant NNX15AM07 G to S.D. We thank Sam Johnson, Justin Gilpin, Michael White, Bronwyn Stephenson and Jordan Herbst for assistance with balloon flights.

Author Disclosure Statement

No competing financial interests exist.

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https://doi.org/10.1017/S1473550416000410 Published online by Cambridge University Press