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REVIEW

Temperature and pH optima of extremely halophilic archaea: a mini-review

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Abstract Archaeal microorganisms that grow optimally at Na^+ concentrations of 1.7 M, or the equivalent of 10% (w/v) NaCl, and greater are considered to be extreme halophiles. This review encompasses extremely halophilic archaea and their growth characteristics with respect to the correlation between the extent of alkaline pH and elevated temperature optima and the extent of salt tolerance. The focus is on poly-extremophiles, i.e., taxa growing optimally at a Na^+ concentration at or above 1.7 M (approximately 10% w/v NaCl); alkaline pH, at or above 8.5; and elevated temperature optima, at or above 50°C. So far, only a very few extreme halophiles that are able to grow optimally under alkaline conditions as well as at elevated temperatures have been isolated. The distribution of extremely halophilic archaea growing optimally at 3.4 M Na^+ (approximately 20% w/v NaCl) is bifurcated with respect to pH optima, either they are neutrophilic, with a pH_{opt} of approximately 7, or strongly alkaliphilic, with pH_{opt} at or above 8.5. Amongst these extreme halophiles which have elevated pH optima, only four taxa have an optimum temperature above 50°C: *Haloarcula quadrata* (52°C), *Haloferax elongans* (53°C), *Haloferax mediterranei* (51°C) and *Natronolimnobius 'aegyptiacus'* (55°C).

Keywords *Archaea* · Halophiles · Poly-extremophiles · Haloalkalithermophiles · Alkaliphiles · Thermophiles

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Extremely halophilic archaea

Recently, a review was conducted of halophilic bacteria that focused upon extremely halophilic bacteria, with Na^+ concentration optima for growth of 1.7 M or greater (Bowers et al. 2009a); the review identified 61 extremely halophilic bacteria. It is commonly known that archaea are inhabitants of some of the most extreme environments on the planet; validly published species include: *Picrophilus torridus* and *Picrophilus oshimae*, which grow at pH values as low as −0.2, and 60°C (Schleper et al. 1995); *Methanopyrus kandleri*, which grows optimally at 110°C, and maximally at 122°C (Kurr et al. 1991, Takei et al. 2008); and *Thermococcus barophilus*, which grows at 40 MPa (Marteinsson et al. 1999). The minimum Na^+ optimum for growth of extreme halophiles is 1.7 M, or the equivalent of 10% NaCl (w/v), as previously defined (Oren 2002; Bowers et al. 2009a). There are numerous species which merely tolerate such sodium concentrations or which grow optimally at marine salt concentrations of approximately 3.5% w/v; however, the authors wish to focus on microorganisms which grow optimally at the upper limits of combined extremes, therefore this review will be limited to extremely halophilic archaea. Using this criterion, 110 extreme halophiles were identified (August 2010) amongst the validly published species of the domain *Archaea* (i.e., published or validated in the *International Journal of Systematic Bacteriology/Systematic and Evolutionary Microbiology*, as listed at http://www.bacterio.cict.fr). In this review Na^+ concentrations are given in mol/liter, rather than % (w/v) NaCl, since some of the alkaliphilic halophiles require media with carbonates, usually provided as sodium carbonates, for pH control (Mesbah et al. 2007a, b; Mesbah and Wiegel 2008). Therefore, the $[Na^+]$ comes both from the sodium carbonates in the media buffer and from the supplemented NaCl. These archaea have been isolated from various and diverse environments such as thalassohaline (i.e., originating from marine waters) solar salterns (Tomlinson et al. 1986); athalassohaline, e.g., Wadi an Natrun, Egypt (Bowers et al. 2009b), and ancient thalassohaline salt lakes, e.g., Great Salt Lake, UT, USA (Paterek and Smith 1988); saline soil (Ihara et al. 1997); and fermented food products (Roh et al. 2007). Unlike the extremely halophilic bacteria, which are relatively evenly distributed between aerobic and anaerobic species, the extremely halophilic archaea are predominately obligate aerobes; out of the 110 species identified (as of August 2010), only 5 species are obligately anaerobic, with the addition of 14 facultative aerobes. The extremely halophilic archaea encompass different types of extrema; for example, *Halorhabdus utahensis*, at 4.6 M, has the highest $[Na^+]$ optimum (Waino et al. 2000); whereas, *Haloferax mediterranei* is capable of growing over the unusually wide $[Na^+]$ range of 1.0–5.2 M (Torreblanca et al. 1986). Additionally, there are eight species, including *Natrialba aegyptia* and *Halobiforma nitratireducens* which tolerate Na^+ saturation (Hezayen et al. 2001, 2002) and *Natronolimnobius 'aegyptiacus'*, which tolerates Na^+ saturation at the highest temperature and alkaline pH (Bowers et al. 2009b).

Many extreme halophiles are mesophilic or neutrophilic; only moderately thermophilic extreme halophiles have been described, with T_{opt} of 50°C or above, along with several alkaliphilic extreme halophiles. Several of the extremely halophilic archaea grow optimally at either alkaline pH (≥8.5) or at elevated temperatures (≥50°C); however, only a very few of the extreme halophiles are able to grow optimally under triply extreme conditions: high $[Na^+]$, alkaline pH, and elevated temperatures. These microorganisms are considered poly-extremophiles (Table 1), if all three conditions are required for optimal growth, as previously described by Bowers et al. (2009a).

Of the 110 validly published, extremely halophilic archaea, no species has a $[Na^+]$ optimum of less than 2 M (approximately 12% w/v NaCl); a stark contrast to the 30% of validly published, extremely halophilic bacteria (Bowers et al. 2009a). Approximately 40% of the extremely halophilic species have published $[Na^+]$ optima equal to or greater than 2.0 M (approximately 12% w/v NaCl) but less than 3.4 M (approximately 20% w/v NaCl), approximately 36% of the species have $[Na^+]$ optima greater than or equal to 3.4 M but less than 4.0 M (approximately 24% w/v NaCl), and approximately 22% (23 species) have published $[Na^+]$ optima of equal to or greater than 4 M. Additionally, approximately 60% of all the extremely halophilic archaea (64 species) tolerate greater than 5 M Na^+ in their growth medium (approximately 29% w/v NaCl), including eight species which tolerate Na^+ saturation, i.e., 5.5–6.5 M, since the saturation point is dependent upon media composition, growth pH and temperature (Table 2). In contrast to the extremely halophilic bacteria, all of the extremely halophilic archaea with published $[Na^+]$ maxima are able to tolerate greater than 4 M Na^+, with the exception of *Methanohalophilus mahii* which has a $[Na^+]$ maximum of 2.5 M (Paterek and Smith 1988).

pH optima and ranges of extremely halophilic archaea

Out of all the validly published extremely halophilic archaea, only 19 have pH optima of 8.5 or greater (Table 2), and therefore are haloalkaliphiles (Table 1), although many of the environments from which these microorganisms were isolated have alkaline pH values (Foti et al. 2008; Mesbah et al. 2007b; Rees et al. 2004). All these species combine an alkaline pH optimum with a $[Na^+]$ optimum of 2 M or greater, and 12 of these species have a $[Na^+]$ optimum of 3.4 M or greater, although only three: *Halorubrum luteum*; *Natronolimnobius 'aegyptiacus'*, a recent

Table 1 Growth characteristics of extremophiles

Growth characteristic	Minimum	Optimum	Maximum
Halotolerant	–	$[Na^+] < 0.2$ M	$[Na^+] > 0.2$ M
Halophilic	$[Na^+] \geq 0.2$ M	$0.2 \text{ M} < [Na^+] < 1.7$ M	–
Extreme	$[Na^+] \geq 0.2$ M	$[Na^+] \geq 1.7$ M	–
Alkalitolerant	pH ≥ 6.0	pH < 8.5	pH > 9.0
Alkaliphilic			
Facultative	pH < 7.5	pH ≥ 8.5	–
Obligate	pH ≥ 7.5	pH ≥ 8.5	pH ≥ 10.0
Thermotolerant	–	$T < 50$°C	$T > 50$°C
Thermophilic	–	$T \geq 50$°C	$T > 55$°C

pH should be measured at optimum growth temperature (Wiegel 1998). 10% added NaCl equals 1.7 M Na^+

– no boundary at this parameter

Table 2 [Na^+], pH and temperature optima and ranges for extremely halophilic archaea

Genus	Species	$Na+_{opt}$	Na+ range	pH_{opt}	pH range	T_{opt}	*T* range	O_2	Isolated from
Haladaptatus	*litoreus*	3.4	1.7–5.1	7	6.0–8.5	38	25–55	Aerobic	Solar saltern, Jiangsu, China
Haladaptatus	*paucihalophilus*	2.9	0.8–5.1	6.3	5.0–7.5	28	25–45	Aerobic	Zodletone Spring, OK
Halalkalicocus	*jeotgali*	2.55	1.7–5.1	7	6.5–9.0	41	21–50	Aerobic	Jeotgal
Halalkalicocus	*tibetensis*	3.4	1.4–5.2	9.8	8.0–9.5	40	23–47	Aerobic	Lake Zabuye, Tibet
Haloarcula	*amylolytica*	3.1	2.0–5.1	7.3	6.5–9.0	41	20–52	Aerobic	Aibi salt lake, China
Haloarcula	*argentinensis*	2.8	2.0–4.5	–	–	40	–	Aerobic	Saltern soil, Argentina
Haloarcula	*hispanica*	3.9	2.5–5.2	7	6.0–8.0	38	25–50	Facultative	Marine salterns, Spain
Haloarcula	*japonica*	3.5	2.5–5.0	7.3	6.0–8.0	44	24–45	Aerobic	Saltern soil, Japan
Haloarcula	*marismortui*	3.7	1.7–5.1	–	–	45	–	Aerobic	Dead Sea
Haloarcula	*quadrata*	3.9	2.7–4.3	6.8	5.9–8.0	52	–55	Anaerobic	Brine pool, Sinai, Egypt
Haloarcula	*vallismortis*	4.3	2.5–	7.5	5.5–8.5	40	20–45	Facultative	Salt pools, Death Valley
Halobacterium	*jilantaiense*	3.3	2.7–5.2	7.3	5.5–8.5	40	22–55	Aerobic	Jilantai salt lake, China
Halobacterium	*noricense*	2.7	2.1–	–	5.2–7.0	41	–	Aerobic	Salt mine, Altausee, Australia
Halobacterium	*piscisalsi*	3.9	2.6–5.1	7.3	5.0–8.0	39	20–60	Aerobic	
Halobaculum	*gomorrense*	2	1.0–	5.5	5.5–8.0	40	–50	Aerobic	Dead Sea
Halobiforma	*haloterrestris*	3.4	2.2–sat.	7.5	6.0–9.2	42	–	Aerobic	Salt soil, Aswan, Egypt
Halobiforma	*lacisalsi*	3.5	1.7–	7.5	6.5–9.0	44	24–57	Facultative	Salt lake, China
Halobiforma	*nitratireducens*	3.5	2.5–sat	8.5	8.0–10.5	39	26–44	Facultative	Chahannao soda lake, China
Halococcus	*dombrowskii*	3.9	2.55–	7	5.8–8.0	39	–	Aerobic	Salt mine, Bad Ischl, Austria
Halococcus	*hamelinensis*	2.55	2.13–5.1	–	4.0–9.0	37	–	Aerobic	Shark Bay, Australia
Halococcus	*morrhuae*	4	2.5–	7.2	5.5–7.9	35	28–50	Aerobic	Various saline waters
Halococcus	*qingdaonensis*	3.06	1.71–	6	4.0–9.0	38	–	Aerobic	Qingdao, China
Halococcus	*saccharolyticus*	4	2.5–	7.2	6.0–8.0	37	–	Aerobic	Marine salterns
Halococcus	*salifodinae*	3.9	2.13–	7	6.8–9.5	40	–	Aerobic	Salt mine, Austria
Halococcus	*thailandensis*	4.2	2.13–	7.4	6.0–10.0	37	15–45	Aerobic	Fish sauce, Thailand
Haloferax	*alexandrinum*	4.25	1.71–sat	7.2	5.5–7.5	37	20–55	Aerobic	Solar saltern, Egypt
Haloferax	*denitrificans*	2.5	1.5–4.5	6.7	6.0–8.0	50	30–55	Facultative	Solar salterns, S.F. Bay, CA
Haloferax	*gibbonsii*	3.5	1.5–5.2	6.8	5.0–8.0	38	15–55	Aerobic	Marine salterns, Spain
Haloferax	*larsenii*	2.8	1.0–4.8	6.8	6.0–8.5	44	25–55	Aerobic	Solar saltern, China
Haloferax	*lucentensis*	4.3	1.7–5.1	7.5	5.0–9.0	37	Oct–45	Aerobic	Alicante, Spain

Table 2 continued

Genus	Species	Na+$_{opt}$	Na+ range	pH$_{opt}$	pH range	T_{opt}	T range	O_2	Isolated from
Haloferax	*mediterranei*	2.5	1.0–5.2	6.5	–	51	20–55	Aerobic	Marine salterns, Spain
Haloferax	*mucosum*	3	1.7–5.1	7.4	6.0–10.0	47	23–55	Aerobic	Shark Bay, Australia
Haloferax	*prahovense*	3.5	2.5–5.2	7.3	6.0–8.5	43	23–51	Aerobic	Telega Lake, Romania
Haloferax	*sulfurifontis*	2.55	1.02–sat	6.6	4.5–9.0	35	18–50	Facultative	Zodletone Spring, OK
Haloferax	*volcanii*	2	1.5–4.5	7	5.0–8.0	45	30–55	Aerobic	Dead Sea
Halogeometricum	*boriquense*	3.9	1.36–	–	–	40	–	Facultative	Cabo Rojo, Puerto Rico
Halogranum	*rubrum*	3.9	2.6–4.3	7.5	6.0–8.0	37	20–50	Aerobic	Solar saltern, Jiangsu, China
Halomicrobium	*katseii*	4.3	3.4–5.2	7.3	6.5–10.0	39	35–50	Aerobic	Lake Tebenquiche, Chile
Halomicrobium	*mukohataei*	3.3	2.5–4.5	–	6.2–8.0	43	–	Facultative	Salt flats, Argentina
Halomicrobium	*mukohataei*	3.3	2.5–4.5	–	–	40	–	Facultative	Argentina
Halopiger	*xanaduensis*	4.3	2.5–5.0	8	–	37	–45	Aerobic	Lake Shangmatala, China
Haloplanus	*natans*	3	2.6–4.3	7	6.5–8.0	40	37–52	Aerobic	Dead Sea water mixture
Haloquadratum	*walsbyi*	3.06	2.38–6.12	–	6.0–8.5	–	25–45	Aerobic	Solar saltern ponds
Halorhabdus	*tiamatea*	4.6	1.71–5.1	6.8	5.5–8.5	45	15–55	Anaerobic	Shaban Deep, Red Sea
Halorhabdus	*utahensis*	4.6	1.5–5.1	6.9	5.5–8.5	50	17–55	Facultative	Great Salt Lake, Utah
Halorubrum	*aidingense*	2.6	1.7–4.3	7.5	7.0–9.0	41	25–52	Aerobic	Aiding Salt Lake, China
Halorubrum	*alkaliphilum*	4.1	1.8–5.2	9	8.0–10.5	38	20–44	Aerobic	Soda lake, Xin-Jiang, China
Halorubrum	*arcis*	4.2	2.2–5.2	7.5	6.0–8.5	42	25–55	Aerobic	Qinghai-Tibet Plateau
Halorubrum	*californiense*	4	2.5–5.0	7.3	6.5–8.5	37	25–42	Aerobic	Cargill Solar Plant, California
Halorubrum	*chaoviator*	4.3	2.0–5.0	7.4	7.0–8.5	37	28–50	Aerobic	Baja California, Mexico
Halorubrum	*coriense*	2.5	2.0–5.2	–	–	50	30–56	Aerobic	Marine salterns
Halorubrum	*distributum*	2.9	1.7–5.2	–	–	41	26–50	Aerobic	
Halorubrum	*ejinorense*	3.4	2.5–5.0	7.5	6.0–10.0	37	25–50	Aerobic	Lake Ejinor, China
Halorubrum	*ezzemoulense*	3.4	2.5–4.3	7.3	6.5–9.0	39	22–50	Aerobic	Ezzemoul Sabkha, Algeria
Halorubrum	*kocurii*	3.4	2.5–	7.5	6.0–9.0	37	25–55	Aerobic	Lake Bagaejinor, China
Halorubrum	*lipolyticum*	2.6	1.7–4.8	7.5	7.0–9.0	47	25–58	Aerobic	Aibi salt lake, Xin-Jiang, China
Halorubrum	*litoreum*	3.4	2.0–5.1	7.3	6.0–9.5	40	20–55	Aerobic	Fuqing solar saltern, China
Halorubrum	*luteum*	4.2	2.5–5.2	9.8	7.5–10.5	35	17–41	Aerobic	Lake Chagannor, China
Halorubrum	*orientale*	3.4	2.5–5.0	8	6.0–10.0	40	25–50	Aerobic	Lake Ejinor, China
Halorubrum	*saccharovorum*	4	1.5–5.2	–	–	50	30–56	Aerobic	

Table 2 continued

Genus	Species	$Na+_{opt}$	Na+ range	pH_{opt}	pH range	T_{opt}	T range	O_2	Isolated from
Halorubrum	*sodomense*	2.1	0.5–	–	–	40	–	Aerobic	Dead Sea
Halorubrum	*tebenquichense*	4.25	2.5–5.2	–	7.0–10.0	40	35–50	Aerobic	Lake Tebenquiche, Chile
Halorubrum	*terrestre*	4.25	2.6–5.1	7.5	5.0–9.0	41	28–50	Aerobic	saline soil
Halorubrum	*tibetense*	3.2	1.7–5.2	9.3	8.0–10.5	39	22–45	Aerobic	Lake Zabuye, Tibet
Halorubrum	*trapanicum*	–	2.5–5.2	–	–	37	–	Aerobic	Trapani salt, Norway
Halorubrum	*vacuolatum*	3.5	2.6–5.1	9.5	8.5–10.5	38	20–50	Aerobic	Lake Magadi, Kenya
Halorubrum	*xinjiangense*	3.3	2.0–5.2	7.3	6.0–10.0	40	Oct–54	Aerobic	Xiao-Er-Kule Lake, China
Halosarcina	*pallida*	3.1	1.7–4.3	6.5	6.0–8.5	30	25–45	Aerobic	Zodletone Spring, OK
Halosimplex	*carlsbadensee*	4.3	3.4–5.1	–	7.0–8.0	38	22–50	Aerobic	Carlsbad, New Mexico
Halostagnicola	*larsenii*	3.4	2.5–5.0	7.5	6.0–9.0	37	25–50	Aerobic	Lake Xilinhot, China
Haloterrigena	*hispanica*	3.4	2.2–4.0	7	6.5–8.5	50	37–60	Aerobic	Fuenta de Piedra Lake, Spain
Haloterrigena	*jeotgali*	3.1	1.7–5.1	7.3	6.5–8.5	41	17–50	Aerobic	Shrimp jeotgal
Haloterrigena	*limicola*	3.1	1.7–5.1	7	6.5–9.0	48	30–61	Aerobic	Aibi salt lake, Xin-Jiang, China
Haloterrigena	*longa*	3.1	1.7–5.1	7.3	6.5–9.0	43	30–56	Aerobic	Aibi salt lake, Xin-Jiang, China
Haloterrigena	*saccharevitans*	3.2	1.71–	7.5	6.5–8.5	44	24–58	Facultative	Aibi salt lake, China
Haloterrigena	*salina*	3.4	2.5–5.0	7.5	6.0–9.0	37	25–50	Aerobic	Lake Xilinhot, China
Haloterrigena	*thermotolerans*	3.3	2.0–	7.3	6.5–8.2	50	–60	Aerobic	Saline crystallizer pond, China
Haloterrigena	*turkmenica*	3.4	2.0–	–	–	45	–	Aerobic	Saline soil, Turkmen
Halovivax	*asiaticus*	3.4	2.5–4.3	7.3	6.0–9.0	37	25–45	Aerobic	Lake Ejinor, China
Halovivax	*ruber*	3.4	2.5–	7.3	6.0–9.0	37	25–45	Aerobic	Lake Xilinhot, China
Methanohalobium	*evestigatum*	4.3	2.6–5.1	7.3	6.0–8.3	48	35–60	Anaerobic	saline lagoons, Sivash
Methanohalophilus	*mahii*	2	0.5–2.5	7.5	–	35	–45	Anaerobic	Great Salt Lake, Utah
Natrialba	*aegyptia*	2.75	1.6–sat	7.5	6.0–9.0	40	–60	Aerobic	Aswan, Egypt
Natrialba	*chahannaoensis*	2.6	1.7–5.1	9	8.5–10.5	45	20–55	Aerobic	Soda lake, China
Natrialba	*hulunbeirensis*	3.4	2.04–5.1	9	8.5–10.5	50	20–55	Aerobic	Soda lake, China
Natrialba	*magadii*	3.5	2.0–5.2	9.5	8.5–11.0	39	20–50	Aerobic	Lake Magadi, Kenya
Natrialba	*taiwanensis*	3.5	2.0–sat.	7.7	5.0–10.0	38	–55	Aerobic	Solar salts, Taiwan
Natrinema	*altunense*	3.7	1.7–	7.4	6.9–8.0	–	–	Facultative	Salt lake, China
Natrinema	*ejinorense*	3.4	1.8–	7	6.0–8.5	37	25–50	Aerobic	Lake Ejinor, China
Natrinema	*gari*	3	1.7–	6.3	5.5–8.5	39	20–60	Facultative	fermented fish sauce

Table 2 continued

Genus	Species	$Na+_{opt}$	Na+ range	pH_{opt}	pH range	T_{opt}	T range	O_2	Isolated from
Natrinema	*pallidum*	3.9	1.7–	7.4	6.0–8.4	39	–	Aerobic	Salted cod
Natrinema	*pellirubrum*	3.9	2.0–	7.6	6.0–8.6	–	20–45	Aerobic	Salted hide
Natrinema	*versiforme*	3.9	1.5–	6.8	6.0–8.0	42	20–53	Facultative	Aibi salt lake, China
Natronobacterium	*gregoryi*	3	2.5–5.2	9.8	8.0–	37	25–40	Aerobic	Rift Valley, Kenya
Natronococcus	*amylolyticus*	3.1	1.46–5.1	9	8.0–10.0	43	22–50	Aerobic	Lake Magadi, Kenya
Natronococcus	*jeotgali*	4.1	1.3-5.1	7.5	7.0–9.5	41	21–50	Aerobic	Fermented shrimp, Korea
Natronococcus	*occultus*	2.8	1.4–5.2	9.8	8.0–	38	25–50	Aerobic	
Natronolimnobius	*'aegyptiacus'*	4.5	2.8–sat	9.5	7.3–10.8	55	31–59	Aerobic	Wadi An Natrun, Egypt
Natronolimnobius	*baerhuensis*	3.4	2.6–	9	7.0–10.0	37	30–46	Aerobic	Soda lakes, China
Natronolimnobius	*innermongolicus*	3.1	1.7–	9.5	7.5–10.0	45	19–54	Aerobic	Soda lakes, China
Natronomonas	*pharaonis*	3.5	2.0–5.2	9	7.0–10.0	37	25–50	Aerobic	Lake Magadi, Kenya
Natronorubrum	*aibiense*	2.9	2.0–4.3	8	6.5–9.5	45	20–50	Aerobic	Aibi salt lake, China
Natronorubrum	*bangense*	3.8	2.0–4.3	9.5	8.0–11.0	45	22–55	Aerobic	Soda lake, Tibet
Natronorubrum	*sediminis*	3.4	2.5–5.0	9	6.0–8.0	37	25–50	Aerobic	Lake Chagannor, China
Natronorubrum	*sulfidifaciens*	3.1	2.1–4.8	9	8.0–10.0	46	20–55	Aerobic	Aiding salt lake, China
Natronorubrum	*tibetense*	3.4	2.0–5.1	9	8.5–11.0	45	22–55	Aerobic	Soda lake, Tibet

For reasons of brevity, citations for the description of the species can be found in the List of Prokaryotic Names with Standing in Nomenclature, http://www.bacterio.cict.fr (updated monthly). Bordered cells indicate data meeting or exceeding one of the following criteria $[Na^+]_{opt} \geq 3.4$ M, $pH_{opt} \geq 8.5$, $T_{opt} \geq 50$°C

– data not found

isolate from the authors; and *Halorubrum alkaliphilum* combine an alkaline pH optimum (9.8, 9.5 and 9.0, respectively) with a $[Na^+]$ optimum of greater than 4 M (4.2, 4.5 and 4.1, respectively) (Hu et al. 2008; Bowers et al. 2009a; Feng et al. 2005). The distribution of the $[Na^+]$ and pH optima of the extremely halophilic archaea (Fig. 1a), display a bifurcated pattern, i.e., with many pH optima near 7, and the others near pH 9. The combinations of pH optimum 7.5 with a $[Na^+]$ optimum of approximately 3.4 M is the most highly represented, followed by the combinations of pH optima 7.3 and 9 with $[Na^+]$ optimum of approximately 3.4 M. This is opposite what was seen upon examination of the extremely halophilic bacteria; in that case, the most highly represented combination was pH 9 and $[Na^+]$ optimum 1.7 (Bowers et al. 2009a). Microorganisms with pH values for optimal growth above 8.5 carry with them the unique energetic problems of alkaliphiles, e.g., an inverted pH gradient and thus a suboptimal proton motive force (Bowers et al. 2009a; Kevbrin et al. 2004; Krulwich et al. 1998). In the case of extremely halophilic alkaliphiles these problems are exacerbated by the need to keep the intracellular sodium concentration below toxic levels, which is frequently as low as a few mM (Mesbah et al. 2009; Oren 2002; Padan and Krulwich 2000). This complication may explain the more prevalent occurrence of microorganisms growing optimally in environments that are pH neutral or near neutral. However, as discussed by Bowers et al. (2009a), this could be due to the fact that researchers may simply have not tried to isolate microorganisms able to thrive under these combined stressors. The biodiversity of the highly alkaline athalassohaline lakes of Wadi An Natrun (North Egypt) is relatively high (Mesbah et al. 2007b), as are the saline habitats of Alexandria, Egypt (Ghozlan et al. 2006) and the various alkaline soda lakes of Rift Valley, Kenya (Duckworth et al. 2006). Currently, the only identified obligately alkaliphilic archaeal species from the lakes of the Wadi An Natrun is *Natronolimnobius 'aegyptiacus'*, which has a pH$^{55°C}$ optimum of 9.5, and grows optimally above 50°C (Bowers et al. 2009b). However, several obligately alkaliphilic archaeal species have been isolated from Lake Magadi and the surrounding Rift Valley, Kenya, including *Natronobacterium gregoryi*, *Halorubrum vacuolatum*, *Natrialba magadii*, *Natronomonas pharaonis*, and *Natronococcus amylolyticus*; each of these microorganisms has a pH optimum of 9.0 or greater (Table 2). As previously recommended (Wiegel 1998), the pH values for *N. 'aegyptiacus'* were measured at the microorganism's optimum growth temperature, denoted with a superscript (i.e., pH$^{55°C}$). The pH measurement of an alkaline, complex growth medium which is at an elevated temperature (i.e., 55°C) with a pH probe calibrated at a much lower temperature (i.e., 25°C) will yield a pH measurement that can be upwards of one unit greater than that measured with a pH probe calibrated at the elevated temperature (Wiegel 1998); this should be considered when comparing the pH optima of alkaliphiles to the pH optima of alkalithermophiles. Three other species are alkalitolerant, and have pH optima of 8.0; of these, only one, *Halopiger xanaduensis*, has a [Na^+] optimum of greater than 4 M (Gutiérrez et al. 2007). With the exception of *Halobiforma nitratireducens*, which is a facultatively aerobic species, all of the extremely halophilic, alkaliphilic archaea are obligate anaerobes (Table 2). This again, is in contrast to the distribution of haloalkaliphilic bacteria, which are relatively evenly distributed between aerobic and anaerobic species.

Temperature optima and ranges of extremely halophilic archaea

The third environmental stressor of interest is elevated temperature. Figure 1b, contrasts in similar fashion to Fig. 1a, the correlation between the temperature optima and the [Na^+] optima of the halophiles under review. Elevated temperature optima—which is, for true thermophiles, above 50°C (Table 2)—are even more infrequent amongst the published extremely halophilic eubacteria than are alkaline pH optima. Only 11 of the extremely halophilic archaea have temperature optima of 50°C or greater, 7 of which are equal to 50°C (Table 2). *Natronolimnobius 'aegyptiacus'* at 55°C has the highest temperature optimum of all the archaeal extreme halophiles; *Haloferax elongans*, *Haloarcula quadrata*, and *Haloferax mediterranei* have temperature optima of 51–53°C (Allen et al. 2008; Oren et al. 1999; Torreblanca et al. 1986). As originally discussed in Vossenberg et al. (1999), at elevated temperature, the cell membrane of thermophilic microorganisms become more permeable to the diffusion of protons and to increased Na^+ diffusion—especially in saline environments—making it more difficult for the cell to keep the intracellular [Na^+] at a millimolar level against the molar extracellular concentration of Na^+, and is possibly a reason that, so far, no hyperthermophilic extreme halophiles have been isolated. The group of extremely halophilic, thermophilic archaea contains one anaerobe, *Haloarcula quadrata* (Torreblanca et al. 1986) and two facultative aerobes, *Halorhabdus utahensis* and *Haloferax denitrificans* (Waino et al. 2000; Tomlinson et al. 1986); all the other microorganisms are aerobic. Fourteen other microorganisms have temperature optima between 45 and 48°C, and are thermotolerant. This group contains two anaerobes, *Methanohalobium evestigatum* and *Halorhabdus tiamatea* (Zhilina and Zavarzin 1987; Antunes et al. 2008), but no facultative aerobes. While there are relatively few truly thermophilic extreme halophiles, many species—approximately 75% of the extreme halophiles with published temperature maxima—are able to tolerate temperatures above 50°C (Table 2). Just over half of the extremely halophilic archaeal species (63%) have temperature optima of 40°C or below: 63% of these species exhibit temperature optima of 38–40°C. The remaining species have temperature optima between 28 and 35°C. It is important to note that not all extremely halophilic species considered have published temperature optima. Interestingly, no strictly psychrophilic ($T_{opt} \leq 15$°C and $T_{max} \leq 20$°C) extreme halophiles have been described to date; however, *Halorubrum lacusprofundi*, despite its optimum growth temperature of 33°C, dominates the archaeal population of Deep Lake, Antarctica, which has a [Na^+] of 3.6–4.8 M and a temperature ranging from −18 to 12°C (Cavicchioli 2006).

Poly-extremophiles: extremely halophilic archaea with concomitant elevated temperature optima and alkaline pH optima

Of all the extremely halophilic archaea, only two species are extremely halophilic, obligately alkaliphilic and thermophilic, and are thus termed poly-extremophiles: *Natrialba hulunbeirensis*, which has a [Na^+] optimum of 3.4 M, a pH optimum of 9.0, and a temperature optimum of 50°C (Xu et al. 2001); and *Natronolimnobius 'aegyptiacus'*, which has a [Na^+] optimum of 4.5, a pH optimum of 9.5 and a temperature optimum of 55°C (Bowers et al. 2009b). Also of note are *Natronorubrum tibetense*, with a [Na^+] optimum of 3.4, a pH optimum of 9, and a thermotolerant temperature optimum of 45°C (Xu et al. 1999) and *Natronorubrum bangense*, with a [Na^+] optimum of 3.8, a pH optimum of 9.5 and a thermotolerant temperature optimum of 45°C (Xu et al. 1999). All four of these

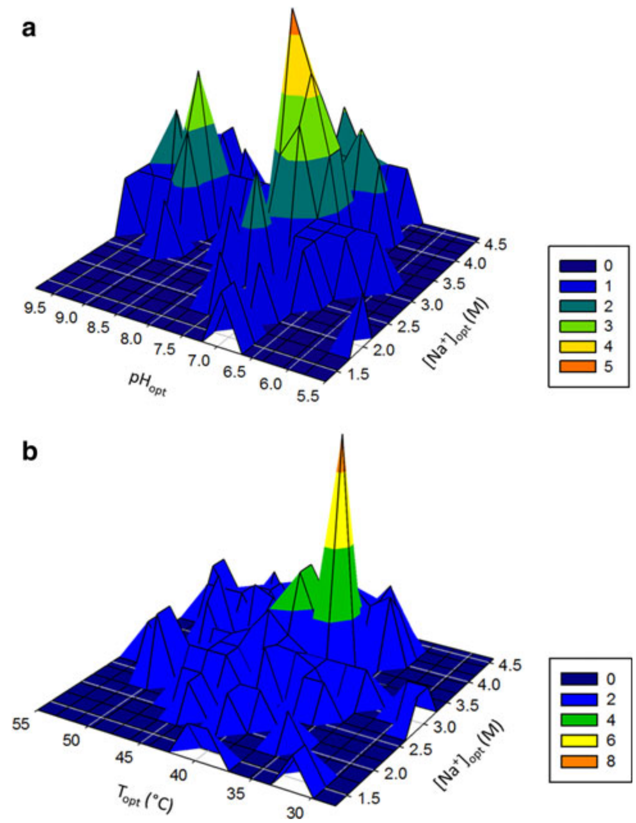

Fig. 1 Correlation of $[Na^{+}]$ optimum and pH or temperature optimum of extremely halophilic archaea. **a** Correlation between $[Na^{+}]$ optimum and pH optimum. $[Na^{+}]$ optimum (*M*) is plotted against pH optimum; number of microorganisms at each locus is plotted on *z*-axis as indicated by *color coding*; no representation (zero) is indicated in the *darkest shade*. **b** Correlation between $[Na^{+}]$ versus temperature optima. $[Na^{+}]$ optimum (*M*) is plotted against temperature optimum (°C); number of microorganisms at each locus is plotted on *z*-axis as indicated by *color coding*; no representation (zero) is indicated in the *darkest shade*

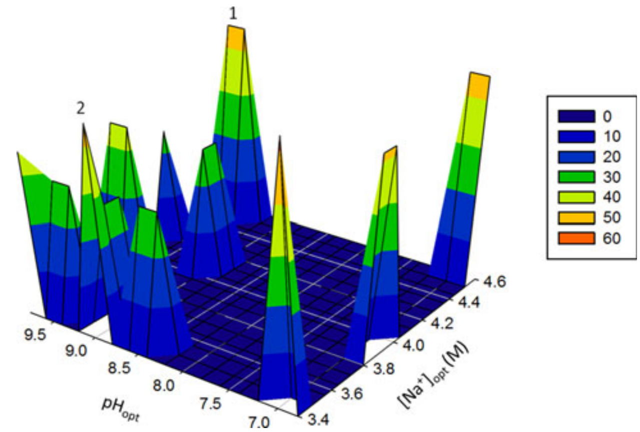

Fig. 2 Clustering of poly-extremophilic archaea relative to other extremely halophilic archaea. Representation of extremely halophilic archaea for which one or both additionally considered growth conditions (pH and temperature) exceed thermophilic and alkaliphilic levels. The peaks representative of the discussed recently isolated poly-extremophiles are labeled numerically, and are delineated below. The *z*-axis *color coding* depicts the temperature optimum of each bacterium. Species represented: *1 Natronolimnobius 'aegyptiacus'*, *2 Natrialba hulunbeirensis*; also represented: *Halalkalicoccus tibetensis*, *Haloarcula quadrata*, *Halobiforma nitratireducens*, *Halorhabdus utahensis*, *Halorubrum alkaliphilum*, *Halorubrum luteum*, *Halorubrum vacuolatum*, *Haloterrigena hispanica*, *Natrialba magadii*, *Natrialba hulunbeirensis*, *Natronolimnobius baerhuensis*, *Natronomonas pharaonis*, *Natronorubrum bangense*, and *Natronorubrum tibetense*; all of which meet two out of three criteria for poly-extremophily

noteworthy species are aerobic (Table 2). These poly-extremophiles cluster uniquely when compared to other known, extremely halophilic archaea (Fig. 2). This is further demonstrated when extremely halophilic species ($Na^{+}_{opt} \geq 3.4$ M) which are also either thermophilic ($T_{opt} \geq 50$°C) or obligately alkaliphilic ($pH_{opt} \geq 8.5$), along with the two identified poly-extremophiles are plotted (Fig. 2). *Natronorubrum bangense*, while nearly unique amongst archaeal species in its poly-extremophily, is neither extraordinarily halophilic nor alkaliphilic. However, on all three axes, $[Na^{+}]$, pH and temperature, *Natronolimnobius 'aegyptiacus'* falls much farther along the axis than do the other extremophiles. The fact that this archaeal microorganism can not only survive but also thrive under these multiple extreme conditions has helped, similarly to the poly-extremophilic bacteria of the order *Natranaerobiales*, to extend the known boundaries for life at a combination of multiple extrema.

Microorganisms living in extreme environments utilize a number of adaptive mechanisms in order to enable them to proliferate, and this is true to an even greater extent of poly-extremophiles. Cytoplasmic acidification for pH adaptation under halophilic growth conditions using multiple monovalent cation/proton antiporters with various pH ranges (Mesbah et al. 2009; Slonczewski et al. 2009), the combined use of organic compatible solutes (da Costa et al. 1998; Roberts 2005) and intracellular accumulation of K^{+} (Mesbah and Wiegel 2008; Oren 2002) for adaptation to osmotic pressure are three of the adaptive mechanisms employed by this group of microorganisms (Oren 2006).

To date, the only anaerobic poly-extremophilic archaea have been isolated from sediment samples taken from one of two locations: solar-heated, alkaline, hypersaline lakes of the Wadi An Natrun, Egypt, with temperatures up to 60°C measured in the salt brine (Bowers et al. 2009a); and an unnamed soda lake in the Hulunbeir Prefecture in China (Xu et al. 2001).

With further investigation into alkaline halobiotic environments more, archaeal poly-extremophiles will likely be isolated and identified. As noted both by Foti et al. (2008) and Mesbah et al. (2007b), few species identified from hypersaline habitats via culture-independent methods are closely related to validly described species. Additionally, in the study performed by Foti et al. (2008) on Russian soda lakes, none of the validly described species were defined as haloalkaliphilic or haloalkalitolerant; however, other studies on Kenyan and Egyptian soda lakes have identified uncultured clones related to validly described haloalkaliphiles (Mesbah et al. 2007b; Rees et al. 2004). While the utility of culture-independent methods cannot be disputed, the presence of a microorganism in an environment does not necessarily imply that the particular environment under investigation represents the optimal environment for the growth of the microorganism. The only way to learn the true ranges and optimum growth conditions for a particular species is to characterize the cultured species, therefore limiting our discussion to validly published microorganisms and *N. 'aegyptiacus'* from the author's laboratory. Although nearly every month novel halophiles are published in the *International Journal of Systematic and Evolutionary Microbiology*, the majority of them from the domain *Archaea*, many grow only at slightly elevated salt concentration, and are not extreme halophiles. The authors hope that this mini-review will encourage other scientists to investigate and characterize other novel poly-extremophiles and further expand the knowledge base of their habitats, limitations and adaptive mechanisms.

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