Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes

Aharon Oren*

Department of Plant and Environmental Sciences, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

*Correspondence: aharon.oren@mail.huji.ac.il

INTRODUCTION

In a study of the proteins of Halobacterium and Halococcus, Reistad (1970) noted an unusual amino acids composition of the cells’ bulk protein: a great excess of the acidic amino acids glutamate and aspartate compared to the basic amino acids lysine and arginine. Analysis of the genome of Halobacterium NRC-1 (Ng et al., 2008) and related organisms (Oren, 2013a) has confirmed the special properties of the proteins of this group of Archaea. The acidic proteins of the Halobacteriaceae typically require high salt concentrations for structural stability and activity, and the presence of such an acidic proteome was considered to be correlated with the accumulation of molar concentrations of KCl to provide osmotic balance to the cells (Lanyi, 1974; Mevarech et al., 2000). With the accumulation of molar concentrations of KCl, an acidic proteome was predicted. However, this is not confirmed by genome analysis. The reported excess of acidic amino acids is due to a high content of Gin and Asn, which yield Glu and Asp upon acid hydrolysis. The closely related Halorhodospira halophila and Halorhodospira halochloris use different strategies to cope with high salt. The first has an acidic proteome and accumulates high KCl concentrations at high salt concentrations; the second does not accumulate KCl and lacks an acidic proteome. Acidic proteomes can be predicted from the genomes of some moderately halophilic aerobes that accumulate organic osmotic solutes (Halomonas elongata, Chromohalobacter salexigens) and some marine bacteria. Based on the information on cultured species it is possible to understand the pI profiles predicted from metagenomic data from hypersaline environments.

Keywords: acidic proteins, osmotic adaptation, halophile, marine bacteria, anaerobic, Halanaerobiaceae

Extremely halophilic microorganisms that accumulate KCl for osmotic balance (the Halobacteriaceae, Salinibacter) have a large excess of acidic amino acids in their proteins. This minireview explores the occurrence of acidic proteomes in halophiles of different physiology and phylogenetic affiliation. For fermentative bacteria of the order Halanaerobiales, known to accumulate KCl, an acidic proteome was predicted. However, this is not confirmed by genome analysis. The reported excess of acidic amino acids is due to a high content of Gin and Asn, which yield Glu and Asp upon acid hydrolysis. The closely related Halorhodospira halophila and Halorhodospira halochloris use different strategies to cope with high salt. The first has an acidic proteome and accumulates high KCl concentrations at high salt concentrations; the second does not accumulate KCl and lacks an acidic proteome. Acidic proteomes can be predicted from the genomes of some moderately halophilic aerobes that accumulate organic osmotic solutes (Halomonas elongata, Chromohalobacter salexigens) and some marine bacteria. Based on the information on cultured species it is possible to understand the pI profiles predicted from metagenomic data from hypersaline environments.

Keywords: acidic proteins, osmotic adaptation, halophile, marine bacteria, anaerobic, Halanaerobiaceae

Figure 1

Figure 1: The distribution of acidic amino acids in proteins of halophiles. The distribution is shown for some of the halophiles used in this study. The median pI value of the proteins encoded by the S. ruber genome is slightly higher than that for Halobacterium NRC-1 (5.03; Figure 1). Salinibacter can be considered as an example of convergent evolution mediated by extensive gene exchange with archaeal halophiles found in the same habitat. The combination

MINI REVIEW ARTICLE

published 05 November 2013
doi: 10.3389/fmicb.2013.00315

www.frontierin.org

November 2013 | Volume 4 | Article 315 | #1
of the "salt-in" strategy and the possession of salt-dependent, highly acidic proteins is thus not necessarily limited to the Halobacteriaceae-lineage of aerobic halophilic Archaea.

THE NATURE OF THE PROTEOME OF THE Halanaerobiales AND OTHER HALOPHILIC ANAEROBES WITHIN THE BACTERIAL DOMAIN

The order Halanaerobiales, families Halanaerobiaceae and Halobacteroidaceae (Rainey et al., 1995) forms a phylogenetically coherent group of anaerobic bacteria affiliated with the low G+C Firmicutes (Kivistö and Karp, 2011; Oren, 2013d). Members of the group have been found in sediments of Great Salt Lake, Utah, the Dead Sea, salterns, oil wells, and in alkaline hypersaline lakes and ponds. Most species grow optimally at 10–15% NaCl, and some tolerate salt up to saturation. Most ferment sugars to acetate, ethanol, H₂, CO₂, and other products. One of these (Halanaerobium orenii, isolated from a warm saline lake in Tunisia), is a true thermophile (up to 68°C), optimum at 60°C) halophile (growth up to 20% NaCl; Cayol et al., 1994). Some genera (Acetohalobium, Natroniella) have a homoacetogenic metabolism. Selenihalanaerobacter shirufu grows by anaerobic respiration with selenate or nitrate as electron acceptor.

Examination of the cytoplasm of members of the Halanaerobiales did not show significant concentrations of organic osmotic solutes (Oren, 1986; Rengpipat et al., 1988), with the possible exception of the finding of glycine betaine in Orenia salinarum grown in media containing yeast extract (Mouné et al., 2000).

However, high ionic concentrations (K⁺, Na⁺, Cl⁻), sufficient to provide osmotic balance, were measured in Halanaerobium orenii (Oren, 1986; Oren et al., 1997), Halanaerobium acetethylicum (Rengpipat et al., 1988), Halobacteroides halobius (Oren, 1986), and Natroniella acetigena (Derkova and Pushkina, 2006). Studies performed on glyceroldehyde-3-phosphate dehydrogenase, NAD-linked alcohol dehydrogenase, pyruvate dehydrogenase, and methyl violoquin-linked hydrogenase from H. acetethylicum (Rengpipat et al., 1988), carbon monoxide dehydrogenase of N. acetigena (Derkova and Boltyanskaya, 2006; Detkova and Pushkina, 2006), the fatty acid synthetase complex of H. praevalens (Oren and Garvecvic, 1993), and hydrogenase and carbon monoxide dehydrogenase of Acetohalobium aransaticum (Zavarzin et al., 1994) showed that all these enzymes function well in the presence of molar salt concentrations, and many need high salt for optimal activity. Therefore the "high-salt-in" strategy was assumed to be the mode of osmotic adaptation in this group (Kivistö and Karp, 2011; Oren, 2013d).

Based on these observations the proteome of the members of the Halanaerobiales was predicted to have a strongly acidic nature. Indeed, analysis of acid hydrolysates of Halanaerobium praevalens, Halanaerobium saccharolyticum, Natroniella acetigena, Halobacteroides halobius, and Sponholobacter lortesti suggested that the bulk protein of all these species may have a strongly acidic nature (Oren, 1986; Derkova and Boltyanskaya, 2006). However, it must be remembered that during acid hydrolysis, the neutral asparagine and glutamine are deaminated to form aspartate and glutamate.

The first evidence against a highly acidic proteome in members of the Halanaerobiales was published in 1987 when it was shown that the H. praevalens ribosomal A-protein is not particularly rich in acidic amino acids (Mattheson et al., 1987). Today genome sequences of three members of the group are available: H. praevalens GSB (Ivanova et al., 2011), a haloadaptaliphile strain from Soap Lake, WA, USA, described as "Halanaerobium hydrogeniformans" (Brown et al., 2011), and Halothermothrix orenii H1685 (Mavromatis et al., 2009). Analysis of these three genomes did not show preferential use of acidic amino acids and no low content of basic amino acids (Elevi Bardavid and Oren, 2012a).

Figure 2. It was earlier suggested that the proteins of H. orenii may lack a pronounced acidic nature as a special adaptation toward growth at high temperatures (Mouts and Patel, 2001), Mavromatis et al., 2009). The properties of the other two genomes show that also the mesophilic species lack an acidic proteome. The bimodal distribution of the pI values with peaks around 4.4–4.8 and 9.8–10.2 is similar to that of the non-halophiles Bacteroides fragilis and Chlorobium tepidum (Mongodin et al., 2005). The main reason for the apparent discrepancy between the bulk protein analyses, showing a pronounced acid nature, and the analysis of the proteins encoded by the genomes, is the high content of glutamine and asparagine, which lose their amide group during the acid hydrolysis procedure involved in sample preparation for amino acid analysis.

Still there is no reason to doubt the presence of high ionic concentrations within the cytoplasm to balance the osmotic pressure of the medium. Analysis of the three Halanaerobiales genomes did not show clear evidence for pathways leading to the synthesis of organic osmotic solutes. A gene for sucrose phosphate synthase was identified in H. orenii, which may point to the possibility of sucrose biosynthesis (Mavromatis et al., 2009). Whether indeed
A renewed study of the special properties of the JW.NM-WN-LFT anaerobic halothermoalkaliphile isolated. Its pI profile (bimodal, with a median pI of 7.47) resembles that possibly higher. Its mode of osmotic adaptation is yet unknown. branch within the Bacteria; it grows between 3 and 10% salt and ing to other phylogenetic lineages were recently sequenced. One the prokaryote world to thrive at high salt concentrations (Elevi may therefore provide new insights into the strategies available to tation in the aerobic halophiles (of acidic, low-pI proteins commonly associated with haloadap- in” strategy of osmotic adaptation but have not adopted the pattern anaerobic halophilic of the be ascertained. The possibility must be taken into account that the sucrose is present in the cells at high concentrations, remains to be ascertained. The possibility must be taken into account that the anaerobic halophilic of the Halanaerobiales group use a “high-salt- in”strategy of osmotic adaptation but have not adopted the pattern of acidic, low-pI proteins commonly associated with haloadapt- in the aerobic halophiles (Halobacteriaceae, Salinibacter). A renewed study of the special properties of the Halanaerobiales may therefore provide new insights into the strategies available to the prokaryote world to thrive at high salt concentrations (Elevi Bardavid and Oren, 2012a).

The genomes of two anaerobic fermentative halophiles belong- ing to other phylogenetic lineages were recently sequenced. One is Flexistipes sinusauricus MAS 10³, isolated from a deep-sea brine pool on the bottom of the Red Sea (Fiala et al., 1990; Lapidos et al., 2011). It was classified as a member of the Deferribacteres, a deep branch within the Bacteria; it grows between 3 and 10% salt and possibly higher. Its mode of osmotic adaptation is yet unknown. Its pI profile (bimodal, with a median pI of 7.47) resembles that of Halanaerobium. The second is Natronanaerobius thermophilus JW.NM-WN-LF³ an anaerobic halomethanotrophic isolated from the Wadi An Natrun lakes in Egypt. It was classified in the newly established order Natronanaerobiales (Clostridiales), requires 3.1–4.9 M NaCl, and is markedly thermophilic (optimum growth at 53°C) and alkalophilic (optimum pH 9.5; Mesbah et al., 2007). Analysis of its genome (Zhao et al., 2011) yielded a markedly acidic proteome (median pI 6.27; Elevi Bardavid and Oren, 2012b; Figure 2). Comparison of the proteins of five anaerobic halophiles of different phylogenetic lineages with different temperature and pH optima thus shows great variations in the acidic nature of the proteome.

**DISPARATE OSMOTIC ADAPTATION STRATEGIES WITHIN THE GENUS Halorhodospira**

The genus Halorhodospira currently contains four species: the type species *H. halophila*, *H. neutriphila*, *H. halochloris* and *H. abdel- maleki*. With respect to salt requirement and tolerance they are quite similar, and all tolerate NaCl at concentrations up to 25% or higher. They can be divided into two groups, phylogenetically separated on the basis of 16S rRNA gene sequences: *H. halophila* and *H. neutriphila* contain bacteriochlorophyll a and carotenoids of the spirilloxanthin group, while *H. halochloris* and *H. abdelmaleki* contain bacteriochlorophyll b and rhodopin carotenoids (Smither and Suling, 1996; Hirschler-Rétif et al., 2005; Oren, 2013e). With respect to their mode of osmotic adaptation they were always considered to be a prime example of organisms that use organic compatible solutes. *H. halophila*, *H. halochloris* and *H. abdelmaleki* were all shown to produce glycine betaine as osmotic solute, with minor amounts of ectoine and trehalose (Truper et al., 1991). Ectoine, now known to be the most widespread osmotic solute in the prokaryote world, was first discovered in *H. halochloris* (Galinski et al., 1985).

In view of their common phylogeny and documented content of organic osmotic solutes, the finding of an acidic proteome and of high intracellular KCl concentrations in *H. halophila* but not in *H. halochloris* (Deole et al., 2013) came as a big surprise. While the latter does not accumulate KCl, the first contains high KCl when grown at high salt (35%) but not at low salt (5%). The genus Halorhodospira thus presents a thus far unique case in which different combinations of KCl concentrations, production of organic osmotic solutes, and presence of acidic vs. non-acidic proteomes are used for osmotic adaptation in phylogenetically closely related species. The authors concluded that “proteome acidity is not driven by stabilizing interactions between K⁺ ions and acidic side chains but by the need for maintaining sufficient solvation and hydration of the protein surface at high salinity . . .”, and they proposed that “obligate protein halophilicity is a non-adaptive property resulting from genetic drift in which constructive neutral evolution progressively incorporates weak K⁺-binding sites on an increasingly acidic protein surface” (Deole et al., 2013).

**ACIDIC PROTEOMES IN MODERATELY HALOPHILIC Gammaproteobacteria**

There is no a priori reason to assume that moderately halophilic aerobic heterotrophic bacteria that synthesize and/or accumulate organic compatible solutes should have a high acidic proteome adapted to function in the presence of high intracellular salt concentrations. A first survey of the proteins of the gammaproteobacterial Chromohalobacter salexigens DSM 3043², based on 238 out of the 3,519 proteins encoded by its genome, indeed showed that most selected proteins were no more acidic than comparable proteins from non-halophilic counterparts. A notable exception was found for periplasmic proteins exposed to the high medium salinity (Oren et al., 2005). Analysis of the entire C. salexigens genome, together with that of the phylogenetically related moderate halophile Halomonas elong- gata IHW³ (Schweibert et al., 2011) showed large peaks of acidic proteins (maximum at pI 4.4–5.0 and 4.5–5.1, respectively) in the pl profiles of the predicted proteins. The median pI values for

![Figure 1](image-url)
the proteins encoded by these genomes are 6.60 and 6.32, respectively (Elevi Bardavid and Oren, 2012b). These values are still in the low pI range, albeit somewhat higher than those reported for high-salt-in organisms such as Halobacterium and Salinibacter (Figure 2). Both organisms synthesize ectoine as compatible solute and accumulate glycine betaine when available in the medium.

Such acidic proteomes are found not only in halophilic and highly halotolerant members of the Gammaproteobacteria, but also in typically marine members of the group. Analysis of the pI distribution of the proteins predicted from the genomes of Alteromonas macroloides ATCC 27126T (Ivans-Martinez et al., 2008), a representative of a genus ubiquitous in the world’s oceans, and of the luminescent Aliivibrio fischeri strain M11 (Mandel et al., 2009) showed a pronounced peak in the acidic range (maximum at pI values of 4.6–4.8), with median pI values of 6.64 and 6.52, respectively (Elevi Bardavid and Oren, 2012b).

**ACIDIC METAPROTEOMES IN HYPERHALINE ENVIRONMENTS**

Metagenomic data from saline and hypersaline environments can be subjected to analyses similar to those shown above for microbial isolates. As shown by Rhodes et al. (2010), there is a general trend of increased average protein acidity (as expressed by the ratio of acidic to basic amino acids) with increased salinity. The highest salinity environments (the Dead Sea, salt-rich crystallizer ponds) have the greatest excess of acidic amino acids in the proteins encoded by the recovered DNA ([Glu + Asp]/([Gly + Arg] + [His]) = 1.42–1.26). This could be expected as high-salt-in strategists (species of Halobacteraeae, Salinibacter) with highly acidic proteomes dominate their biota. Metagenomes from different samples from the marine environment gave values in the range 0.86–0.95, and the benthic microbial mats in the 9% salt lagoons of Guerrero Negro, Mexico (Kunin et al., 2008), yielded an intermediate value of 1.01 on the average.

The finding that the pI distribution of the proteins encoded by the metagenome of the Guerrero Negro microbial mats showed an acid-shifted proteome (major peak at pI 4.5–4.9, median pI 6.8) as compared to non-halophilic or marine environments was at first puzzling, as at that salinity microorganisms are expected to exclude salt from their cytoplasm to a large extent (Elevi Bardavid and Oren, 2012b). On the other hand, the analysis of the genomes of different anaerobic halophiles (Halanaerobiales and others) unexpectedly failed to show a highly acidic proteome (Elevi Bardavid and Oren, 2012a). The case of the two Halorhodospira species demonstrates that phylogenetically very closely related organisms may use completely different strategies for osmotic adaptation, and accordingly have highly different amino acid signatures of their proteins. The more or less coherent picture of a clear correlation between phylogenetic affiliation and modes of salt adaptation that was apparent in the past (Trüper et al., 1991; Oren, 2008) needs to be evaluated in view of the above-mentioned observation that some typically marine Gammaproteobacteria (Alteromonas and Vibrio spp.) are rich in low-pI proteins. A possible explanation is that other, possibly very abundant marine bacteria show the opposite trend. The small genome of “Pelagibacter ubique” (Alphaproteobacteria, the “SAR-11” phylotype, Giovannoni et al., 2005) encodes for 1,393 proteins with an overall excess of 2% ([Arg + Arg] + [Glu + Asp]). For comparison, Halobacterium, Salinibacter and H. elongata and Halomonas all show an excess of acidic amino acids (7.5, 4.1, and 2.8 mol %, respectively; Elevi Bardavid and Oren, 2012b). Most “Pelagibacter” proteins have pI values between 9.4 and 10.8, with a median value of 8.42.

**FINAL COMMENTS**

The genomic and metagenomic data discussed above show that dominance of acidic proteins in halophilic microorganisms is by no means restricted to the Halobacteraeae and to Salinibacter which resembles the Halobacteraeae in many properties. Somewhat less acidic proteomes are found in many moderately halophilic and even in some marine bacteria, organisms that exclude salt from their cytoplasm to a large extent (Elevi Bardavid and Oren, 2012b). The question whether the environment for primordial life may have been hypersaline has been addressed earlier (Dundas, 1998). Therefore the issues discussed above may even have direct implications for our ideas on the origin of life and the properties of the earliest organisms that inhabited our planet.

**ACKNOWLEDGMENTS**

I thank Rahel Elevi Bardavid and Omri Finkel for their contributions to the data evaluation. This study was supported by grants no. 1103/10 and 343/13 from the Israel Science Foundation.
Oren Acidic proteins in halophilic microorganisms

Brown, S. D., Begemann, M. B., Y. V. (2006). Relationships between pH and fermentative metabolism in Halobacteroidaceae. Int. J. Syst. Evol. Microbiol. 56, 1083–1090. doi: 10.1099/ijs.0.64708-0

Galinski, E. A. (2013). The family Halobacteriaceae. In: The Prokaryotes. Fourth Edition, eds E. Rosenberg, E. F. DeLong, J. L. Stackebrandt, E. C. Staley, R. E. Bond, and J. T. 0. mol (Berlin, Heidelberg: Springer). pp. 21–129. doi: 10.1007/978-3-642-34325-0

Galinski, E. A. (1995). Osmoadaptation in bacteria. Adv. Microb. Physiol. 37, 273–328.

Kamin, V., Raes, J., Harris, J. K., Spurz, J. R., Walker, J. I., Varner, N., et al. (2008). Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. ISME J. 2, 375–377. doi: 10.1038/ismej.2008.35

Lamy, J. K. (1974). Salt-dependent properties of proteins from extremely halophilic bacteria. Bacteriol. Rev. 38, 279–299.

Lapidus, A., Cheriotis, O., Nolan, M., Lucas, S., Hammon, N., Doherty, S., et al. (2011). Genome sequencing of the moderately thermophile Halomonas asburiae strain (MAS10T). ISME J. 5, 1. doi: 10.1038/ismej.2010.54

Longo, I. M., Lee, J., and Huber, M. (2013). Simplified protein design biased for brine pH yields a foldable, halophilic protein. Proc. Natl. Acad. Sci. U.S.A. 110, 2135–2140. doi: 10.1073/pnas.1219510110

Mandel, M. I., Wolkowski, M. S., Stahl, E. V., Vainik, K. L., and Ruby, E. G. (2009). A single regulatory gene is sufficient to alter bacterial host range. Nature 458, 215–218. doi: 10.1038/nature0760

Mikrobiologiya 75, 5–17. doi: 10.1002/0471142727.mb1921. doi: 10.1002/0471142727.mb1921

Stedman, K. J., Hirschler, A., Hammer, H., Caron, P., and Oren, A. (2008). Diversity of halophilic microorganisms: environments, phylogeny, and applications. J. Ind. Microbiol. Biotechnol. 35, 86–96. doi: 10.1007/s10292-007-9212-9

Oren, A. (2008). Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Salinity Sci. 4, 2. doi: 10.1007/s11186-008-9042-2

Oren, A. (2011). “Diversity of halophilic life,” in Halophiles in Extreme Environments. Edn, eds E. Rosenberg, E. F. DeLong, J. L. Stackebrandt, E. C. Staley, R. E. Bond, and J. T. O. mol (Berlin, Heidelberg: Springer). pp. 309–325.

Oren, A. (2013a). “The family Halobacteriaceae,” in The Prokaryotes: An Atlas for the 21st Century. Handbuch der Biologie der Bakterien: Ecophysiology and Biochemistry, 4th Edn, eds E. Rosenberg, E. F. DeLong, E. Thompson, S. Loy, and E. Stackebrandt (New York, NY: Springer), in press.

Oren, A. (2013b). “Life at high salt concentrations,” in The Prokaryotes: An Atlas for the 21st Century. Handbuch der Biologie der Bakterien: Ecophysiology and Biochemistry, 4th Edn, eds E. Rosenberg, E. F. DeLong, E. Thompson, S. Loy, and E. Stackebrandt (New York, NY: Springer), in press.

Oren, A. (2013c). Halobacterium, an extremely halophilic bacterium with archival properties. EEM Microbiol. Lett. 342, 1–9.

Oren, A. (2015b). “The order Halobacteriales, families Halobacteraceae and Halobacterales,” in The
Oren, A. (2013e). “Family Ectothiorhodospiraceae,” in The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology and Biochemistry, 4th Edn, eds E. Rosenberg, E. F. DeLong, F. Thompson, S. Lory, and E. Stackebrandt (New York, NY: Springer), in press.

Oren, A., and Gurevich, P. (1993). Oren, A., Heldal, M., Norland, S., and Oren, A., Heldal, M., and Norland, S. (1997). X-ray microanalyses of intracellular ions in the anaerobic halophilic sulfur bacterium Haloarcula marismortui. Curr. J. Microbiol. 43, 508–512. doi: 10.1139/03-005.

Oren, A., Heldal, M., Norland, S., and Galinski, E. A. (2002). Intracellular ion and organic solute concentrations of the extremely halophilic bacterium Salinibacter ruber. Extremophiles 6, 495–498. doi: 10.1007/s00792-002-0206-5.

Oren, A., Larimer, F., Richardson, P., Lapidus, A., and Csonka, L. N. (2003). How to be moderately halophilic with a broad salt tolerance: clues from the genome of Chromohalobacter salexigens. Extremophiles 7, 275–279. doi: 10.1007/s00792-003-0462-7.

Oren, A., and Mana, L. (2002). Amino acid composition of bulk protein and salt relationships of selected enzymes of Salinibacter ruber, an extremely halophilic bacterium. Extremophiles 6, 217–223. doi: 10.1007/s00792-001-0241.

Rainey, F. A., Zhilina, T. N., Boullygina, E. S., Stackebrandt, E., Tourou, T. P., and Zavarzin, G. A. (1995). The taxonomic status of the fermentative halophilic anaerobic bacteria description of Halobacteroides salinarum nov., Halobacteroides firmum nov., Oceua gen. nov. and further taxonomic rearrangements in the genus and species level. Anaerobe 1, 185–190. doi: 10.1006/anab.1995.1018.

Riosdahl, R. (1970). On the composition and nature of the bulk protein of extremely halophilic bacteria. Arch. Mikrobiol. 71, 355–360. doi: 10.1007/BF00473731.

Bengtsson, S., Lowe, S. E., and Zeikus, J. G. (1988). Effect of extreme salt concentrations on the physiology and biochemistry of Halobacteroides acetogenicus cells. J. Bacteriol. 170, 3065–3071.

Rhodes, M. E., Fire-Gibbon, S., Oren, A., and House, C. H. (2010). Amino acid signatures of salinity on an environmental scale with a focus on the Dead Sea. Extrem. Microbiol. 12, 2613–2625. doi: 10.1111/j.1462-2920.2010.02323.x.

Schwibbert, K., Martin-Sanguino, A., Bagran, I., Hiedrich, G., Lattion, G., Setz, H., et al. (2011). A blueprint of extreme metabolism from the genome of the industrial producer Halomonas DS40. Environ. Microbiol. 13, 1957–1968. doi: 10.1111/j.1462-2920.2010.02500.x.

Trüper, H. G., Severin, I., Welzl, A., Müller, E., and Galinski, E. A. (1991). “Halophile, taxonomic phylogeny and nomenclature,” in General and Applied Aspects of Halophilism, ed F. Rodriguez-Valera (New York, NY: Plenum Press), 3–7.

Vaneian, N., and Oren, A. (2009). Salinibacter longsea gen. nov., sp. nov., a red halophilic member of the Rhodospirilales. Int. J. Syst. Evol. Microbiol. 59, 2571–2574. doi: 10.1099/ijs.0.016592-0.

Zavarzin, G. A., Zhilina, T. A., and Paskova, M. A. (1994). “Halophilic autotrophic bacteria,” in Aequit den, ed H. L. Drake (New York: Chapman & Hall), 432–444.

Zhao, B., Mesbah, N. M., Dalin, E., Gooden, L., Nolan, M., Paskova, S., et al. (2011). Complete genome sequenced of the anaerobic, halophilic alkalithermophile Natronoveriscus thermophilus. J. Bacteriol. 193, 4023–4024. doi: 10.1128/JB.00573-11.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 June 2013; paper pending publication: 06 October 2013; accepted: 06 October 2013; published online: 05 November 2013.

Citations: Oren A (2013). Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes. Front. Microbiol. 4:215. doi: 10.3389/fmicb.2013.00215.

This article was submitted to Extreme Microbiology, a section of the journal Microbiology. Copyright © 2013 Oren. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided that the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.