An Extremely Halophilic Proteobacterium Combines a Highly Acidic Proteome with a Low Cytoplasmic Potassium Content*§

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Background: The molecular cause and adaptive advantage of proteome halophilicity and acidity in haloarchaea accumulating KCl for osmoprotection are unresolved.

Results: Halorhodospira halophila has an acidic proteome and accumulates molar concentrations of KCl but only when grown in hypersaline medium.

Conclusion: KCl accumulation occurs in Proteobacteria and does not necessitate proteome halophilicity.

Significance: Proteome acidity is needed for protein surface hydration; obligate proteome halophilicity results from constructive neutral evolution.

Halophilic archaea accumulate molar concentrations of KCl in their cytoplasm as an osmoprotectant and have evolved highly acidic proteomes that function only at high salinity. We examined osmoprotection in the photosynthetic Proteobacteria Halorhodospira halophila and Halorhodospira halochloris. Genome sequencing and isoelectric focusing gel electrophoresis showed that the proteome of H. halophila is acidic. In line with this finding, H. halophila accumulated molar concentrations of KCl when grown in high salt medium as detected by x-ray microanalysis and plasma emission spectrometry. This result extends the taxonomic range of organisms using KCl as a main osmoprotectant to the Proteobacteria. The closely related organism H. halochloris does not exhibit an acidic proteome, matching its inability to accumulate K+. This observation indicates recent evolutionary changes in the osmoprotection strategy of these organisms. Upon growth of H. halophila in low salt medium, its cytoplasmic K+ content matches that of Escherichia coli, revealing an acidic proteome that can function in the absence of high cytoplasmic salt concentrations. These findings necessitate a reassessment of two central aspects of theories for understanding extreme halophiles. First, we conclude 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 through strongly hydrated carboxylates. Second, we propose that obligate protein halophilicity is a non-adaptive property resulting from genetic drift in which constructive neutral evolution progressively incorporates weakly stabilizing K+-binding sites on an increasingly acidic protein surface.

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Approximately 97% of all water on earth is present in saline oceans, saline lakes, inland seas, and saline groundwater (1), and roughly one-quarter of the land on earth is underlain by salt deposits (2). Thus, saline and hypersaline environments are highly abundant and of great ecological significance. In addition, salinity is a major determinant for microbial community composition (3). Therefore, halophilic adaptations are of general biological interest. Most extreme halophiles are members of the Halobacteria (Archaea), and particularly Haloarchaeum salinarum has been studied extensively (4). Extreme halophilicity in bacteria is less well studied but has been described for the chemotroph Salinibacter ruber and the photosynthetic purple bacterium Halorhodospira halophila (5, 6). A key factor in the halophilic adaptations of H. salinarum and S. ruber is that they accumulate up to 5 M KCl in their cytoplasm (7–11). This osmoprotection strategy results in unidentified protein-solvent interactions that drive a proteome-wide adaptation in which all proteins have an acidic isoelectric point due to an excess of Glu and Asp residues (4, 12–16). The taxonomic distribution of the reported use of KCl as a major osmoprotectant in extreme halophiles is quite limited: it has been reported only in Halobacteria, in S. ruber (Bacteroidetes), and, to a somewhat lesser extent, in the Halanaerobiales (Firmicutes) (8, 9).

Many enzymes from extreme halophiles with acidic proteomes require the presence of at least 1 M salt to be stable and active (12–14). Stabilizing interactions between K+ ions and the negatively charged protein surface are thought to cause the obligately halophilic nature of these enzymes (12–14, 16, 17). Thus, the functioning of both of these organisms and the proteins that they contain requires the presence of salt. Enzyme halophilicity necessitates the organism to maintain a high cytoplasmic salt concentration and is likely to be a factor in the minimum requirement of ~15% NaCl for growth of H. salinarum and S. ruber (4, 15). Although the adaptive value and molecular basis for obligate protein halophilicity have attracted significant attention (12–14, 16), the molecular interactions...
involved remain elusive. The emerging view is that the use of KCl as an osmoprotectant, proteome acidity, and obligate proteome halophilicity are connected through a causal chain of events. In this interpretation, the use of KCl as an osmoprotectant requires stabilizing interactions between K⁺ ions and acidic side chains on the protein surface, and protein halophilicity is a consequence of the resulting acidic nature of proteomes in extreme halophiles. Obligate protein halophilicity would thus be an obligatory “price to pay” for retaining protein function in a cytoplasm that contains molar concentrations of KCl. Here, we present experimental results that challenge this view.

EXPERIMENTAL PROCEDURES

Cell Growth—H. halophila SL1 and H. halochloris were grown in DSMZ 253 medium without yeast extract containing different salt concentrations (3.5–35% NaCl). H. salinarum was grown in DSMZ 227 medium. Escherichia coli B (ATCC 11303) was grown in 5 g/liter nutrient broth. The cultures were grown until late exponential phase and harvested at A₆₀₀ = 1–1.2. For the determination of growth curves, cell cultures were grown in triplicates in three independent experiments, for a total of nine measurements per data point.

Electron Microprobe Analysis—H. halophila, H. halochloris, H. salinarum, and E. coli cultures were pelleted, washed with isotonic NaCl or ammonium sulfate solutions, again pelleted, and spread on a carbon planchet. The planchet was immediately plunged into isopentane chilled in liquid nitrogen at −150 °C. The preparation was then transferred to a vacuum pump, and the sublimed water vapor was removed. The freeze-dried cells were exposed to anhydrous paraformaldehyde vapor. The samples were examined in an FEI Quanta 600 field emission gun environmental scanning electron microscope vapor. The samples were examined in an FEI Quanta 600 field emission spectrometry (Spectro Arcos). The scanning electron microscope was operated at an accelerating voltage of 20 kV, and the gun current was 50–55 μA. The analysis was confined to a field of overlaying organisms at a magnification of ×5000 and covering an estimated area of 1–2 μm². Counts were accumulated over 180 s. The measurements were performed in two independent experiments with measurements at five different sample areas, for a total of 10 measurements for each cell sample.

Determination of Cellular Potassium and Chloride Content—For plasma emission spectrometry, 20 ml of cell culture was centrifuged. Cell pellets were suspended in isotonic ammonium sulfate solutions, pelleted, and dried for 48 h at 65 °C. Dried cell pellets were divided in two halves. The first half was used to measure potassium and sodium by inductively coupled plasma emission spectrometry (Spectro Arcos). The second half was used for the colorimetric determination of chloride using a Lachat 8000 QuickChem flow injection analyzer. The measurements were performed in triplicates in two independent experiments, for a total of six measurements per data point. To calculate cytoplasmic K⁺ and Cl⁻ concentrations for H. halophila, H. halochloris, and H. salinarum, measurements on E. coli samples were performed in parallel and were used as a standard to calibrate the measured amounts of K⁺ and Cl⁻.

This approach uses the published cytoplasmic K⁺ and Cl⁻ concentrations of E. coli of 211 and 188 mM, respectively (18).

Isoelectric Focusing Gel Electrophoresis—H. halophila and H. halochloris cells were harvested during exponential growth. Cultures with an A value at 660 nm near 1 were centrifuged, and the cell pellets were resuspended in 200 μl of 20 mM Tris buffer (pH 6.8), placed on ice, and sonicated (three times for 10 s). The supernatants were dialyzed in Slide-A-Lyzer dialysis cassettes. The volume of the samples was adjusted with lysis buffer to equal amounts of cell material based on their original A₆₆₀. The samples were then TCA-precipitated using ice-cold acetic acid and trichloroacetic acid. The resulting pellet was resuspended in urea buffer, and the supernatant was used for isoelectric focusing gel electrophoresis using a Mini-PROTEAN apparatus at 500 V and Bio-Rad IEF precast gels. Two different IEF markers (Bio-Rad IEF standard, broad range of pI 4.45–9.6; and Serva Liquid Mix, pI 3–10) were used at 5–10 μg/lane. The gels were stained with Coomassie Blue R-250.

Genomics and Bioinformatics—The genome of H. halophila was determined by shotgun sequencing in collaboration with the Joint Genome Institute. The pl values of all proteins encoded in the proteomes of H. halophila, S. ruber, H. salinarum, Haloarcula marismortui, Natronomonas pharaonis, Nitrosococcus oceani, E. coli, and Rhodobacter sphaeroides were calculated as reported previously (19). Putative H. halophila K⁺ transporters identified in the Integrated Microbial Genomes System (20) were used as query sequences in BLAST searches of the Transporter Classification Database (21) and GenBank. To identify additional K⁺ transporters that may have been missed in the initial annotation, literature searches were performed to compile a list of possible K⁺ transporter system and associated genes. The protein sequences of these were obtained from GenBank and used as queries in BLAST searches of the H. halophila genome. All BLAST searches were performed using the default expect value (E-value) of 10. All H. halophila hits to each query were screened by manual inspection of bit score, E-value, and alignment length to determine sequence similarity to the query. An E-value below 10⁻⁵ and an alignment length of ~75% or greater were used as threshold criteria to identify H. halophila candidate K⁺ transporter genes. The domains represented in the protein translations of the candidate gene sequences were examined using the NCBI Conserved Domain Search.

RESULTS AND DISCUSSION

The Proteome of H. halophila Is Strongly Acidic—Here, we examined the osmoprotection strategy of two closely related organisms, H. halophila and H. halochloris (5, 22). These obligately anaerobic and phototrophic organisms exhibit growth over an unusually wide range of medium NaCl concentrations and are capable of growth down to 3.5% NaCl (the salinity of sea water) (Fig. 1). Whereas H. halochloris exhibits a growth optimum from ~15 to 25% NaCl, H. halophila grows optimally at all NaCl concentrations above 15%.

To explore halophilic adaptations in H. halophila, we determined and analyzed the DNA sequence of its genome. The completed genome sequence of H. halophila SL1 (NCBI reference sequence NC_008789.1) was assembled into a single cir-
cular contig based on 36,035 reads, with an average of 12-fold sequence coverage per base and an error rate of 1 in 100,000. The *H. halophila* genome consists of a single circular chromosome that is 2.7 megabases in length and that encodes 2493 predicted genes. For 1905 of protein-coding genes, putative function could be assigned.

Because halophiles that use KCl as a main osmoprotectant often exhibit proteome acidity, we compared the distribution of the predicted isoelectric points for all proteins in *H. halophila* to the pI distributions of extreme halophiles known to utilize K⁺ as an osmoprotectant (*H. salinarum*, *H. marismortui*, and *N. pharaonis*) and three non-halophilic Proteobacteria (*N. oceanii*, *E. coli*, and the purple phototroph *R. sphaeroides*) and three non-halophilic Proteobacteria (*N. oceanii*, *E. coli*, and the purple phototroph *R. sphaeroides*). All three taxonomically fairly closely related to *H. halophila*. The pI distributions were normalized to the same total number of proteins. We observed a strong predominance of acidic proteins, with a maximum of the acidic peak at pH 5.3 (Fig. 2a). This analysis therefore revealed that *H. halophila* has a highly acidic proteome.

The calculated proteome pI distributions do not consider the different expression levels of proteins or possible post-translational modifications. To experimentally verify the pI distributions derived through bioinformatics, we examined total cell extracts from *E. coli*, *H. salinarum*, and *R. sphaeroides* grown at 35% NaCl. Lane 1, 5, and 7 contain the indicated pI markers.

FIGURE 1. Effect of salt concentration on the growth of *H. halophila* (●) and *H. halochloris* (■). The dependence of the doubling time (a) and final absorbance (b) on the salinity of the growth medium is shown. c, typical growth curves of *H. halophila* at 5% (●), 15% (■), and 35% (▲) NaCl.

FIGURE 2. *H. halophila* has an acidic proteome. a, proteomic distribution of calculated pI values for *H. halophila* (gray trace) and *S. ruber* (black trace) compared with three extremely halophilic halobacteria known to utilize K⁺ as an osmoprotectant (*H. salinarum*, *H. marismortui*, and *N. pharaonis*) and three non-halophilic Proteobacteria (*N. oceanii*, *E. coli*, and the purple phototroph *R. sphaeroides*). The pI distributions were normalized to the same total number of proteins. b, isoelectric focusing gel electrophoresis of total cell extracts from *E. coli* (lane 2), *H. salinarum* (lane 3), *H. halophila* (lane 4), and *H. halochloris* (lane 6) grown at 35% NaCl. Lane 1, 5, and 7 contain the indicated pI markers.
as their major osmoregulntant, we predicted the same halophilic strategy in H. halophila.

H. halophila Accumulates Molar Amounts of KCl—To test the expectation that H. halophila accumulates high concentrations of KCl, we examined its cytoplasmic ionic composition. Cells grown at 35% NaCl were investigated using x-ray microanalysis, together with E. coli and H. salinarum cells for comparison. A prominent K⁺ peak was observed in both the H. halophila and H. salinarum samples, whereas this peak was very small in the E. coli cell material (Fig. 3a), indicating a high degree of K⁺ accumulation in H. halophila. Similarly, prominent Cl⁻ signals were detected in the two extremely halophilic microorganisms.

To quantify these observations, we performed inductively coupled plasma emission spectrometry measurements. These experiments confirmed that the cytoplasmic K⁺ content of H. halophila is high. Comparison with E. coli cell material indicated a cytoplasmic concentration of 2.1 ± 0.1 mM in H. halophila under the growth conditions used (Fig. 3b). For comparison, a typical cytoplasmic K⁺ concentration in E. coli is 211 mM (18). Cellular Cl⁻ levels were determined colorimetrically, indicating the presence of 3.3 ± 0.4 mM in H. halophila. These results show that K⁺ is a major osmoregulntant in H. halophila, with Cl⁻ being the major counterion, extending the use of KCl as a major osmoregulntant from the select group of the Halobacteroides (S. ruber), and Halanaerobiales to the Gammaproteobacteria. The highest reported cytoplasmic salt concentrations in these organisms are 1.9 mM for Halanaerobium praevalens (8) and near 5.0 mM for H. salinarum and S. ruber (9, 10).

In these measurements, we assumed that the cytoplasmic volumes of E. coli, H. salinarum, H. halophila, and H. halochloris cells/g of dried cell material are comparable. In view of the known technical difficulties associated with the accurate experimental determination of the cytoplasmic volume of bacteria (23), this approach would not be expected to significantly reduce the accuracy of the reported cytoplasmic solute concentrations. This assumption is validated by two experimental observations. First, this approach yields values for the cytoplasmic concentration of these ions in H. salinarum that are consistent with published values. Second, the values derived from this approach for both H. halophila and H. halochloris (see below) match the elemental composition as determined by electron microprobe analysis.

In line with its capability to accumulate K⁺, the H. halophila genome encodes two specific K⁺ transport systems (Table 1 and supplemental Table 1): a K⁺/H⁺ antiporter and a Trk K⁺ uptake system. The H. halophila genome also harbors 10 genes encoding cation transporters likely to be involved in K⁺ or Na⁺ transport. Comparison with the genomes of the organisms listed in Fig. 2a (supplemental Table 2) revealed that most of the genes related to K⁺ and Na⁺ transport in H. halophila are also present in both the non-halophilic taxonomic relatives of H. halophila, in S. ruber, and in the haloarchaea. Further experimental work is needed to identify which of these genes are critical for osmoregulation in H. halophila.

Osmoprotection in H. halochloris—In contrast with the situation in H. halophila, both x-ray microanalysis and plasma emission spectrometry revealed that H. halochloris does not accumulate KCl beyond levels found in E. coli (Fig. 3). This result is matched by the broad distribution of pl values of its proteome as revealed by isoelectric focusing gel electrophoresis (Fig. 2b). Interestingly, H. halochloris has been reported to use the organic osmolyte glycine betaine as its main osmoregulntant (24).

The striking difference in the cytoplasmic KCl content and the matching proteome acidity of H. halophila and H. halochloris.
Prototypic Halophiles exhibit a high KCl content
sis indicate a quite recent and strong divergence in osmoprotectant strategy. A similar recent change in osmoprotectant strategy is suggested by the absence of a strongly acidified proteome in Halorhodospira orollis (25), whereas closely related members of the Halanaerobiales have an acidic proteome and utilize K⁺ as an osmoprotectant (8).

Osmoregulation of KCl Accumulation in H. halophila—We next examined the dependence of the cellular KCl content of H. halophila on the NaCl concentration of the growth medium. This revealed that the cytoplasmic K⁺ concentration of H. halophila is strongly regulated by the salinity of the growth medium: an ~5-fold reduction in cellular K⁺ content (from 2.1 to 0.4 M) was observed upon a decrease in medium NaCl concentration from 35 to 5% (Fig. 4a). Similarly, the cellular Cl⁻ concentration was reduced by ~6-fold (from 3.3 to 0.5 M). These observations were confirmed by x-ray microanalysis (Fig. 4b). Thus, upon growth in medium containing 5% NaCl, the cytoplasmic K⁺ concentration of H. halophila is very similar to that of E. coli.

Because it would be highly unexpected for an organism to contain an acidic proteome in the absence of a high cytoplasmic KCl concentration, we considered the possibility that growth of H. halophila at reduced NaCl concentrations greatly reduces its proteome acidity through regulation of gene expression. However, isoelectric focusing gel electrophoresis revealed that even upon growth in medium containing 5% NaCl, the proteome of H. halophila remained strongly acidic (Fig. 4c). These results show that although H. halophila possesses a strongly acidic proteome, it is capable of reducing its cellular K⁺ concentration to low values.

The above x-ray microanalysis experiments (Figs. 3a and 4b) were performed using cells washed with isotonic ammonium sulfate solutions to allow the detection of both K⁺ and Cl⁻ without contributions of ions from the growth medium to the detected signals. To ensure that this washing procedure did not disrupt cell integrity and thus perturb the measurement of the elemental composition of the cytoplasm, we performed x-ray microanalysis on cells washed with isotonic solutions of either NaCl or ammonium sulfate (Fig. 5). For both washing solutions, the same pattern was observed with respect to the K⁺ content of the samples, providing direct experimental evidence that washing with ammonium sulfate does not perturb the integrity of the cells. As expected, all cell samples washed with NaCl consistently revealed a high content of sodium and chloride, precluding the experimental examination of the cellular Cl⁻ content. For cells washed with ammonium sulfate, the sodium content was greatly reduced concomitant with a strong increase in sulfur content. These data indicate that ions in the washing solution strongly contribute to the detected elemental composition of the cells as studied by electron microprobe analysis. Cells washed with ammonium sulfate allow the detection of changes in both cellular chloride and potassium content.

Proteome Acidity to Maintain Protein Hydration—The current model of halophilic adaptations is that the use of KCl as a main osmoprotectant is an energetically attractive strategy because it does not involve the biosynthesis of molar concentrations of organic osmoprotectants (7). This strategy requires that the organism adapts all of its cytoplasmic components to the presence of high KCl concentrations. These changes involve the strong acidification of the entire proteome, with an excess of Glu and Asp residues specifically on the surface (26) of nearly all proteins. It is believed that this acidification results in the obligately halophilic nature of the enzymes from extreme halophiles (12, 13, 16, 17) and therefore necessitates the perma-

FIGURE 4. Effect of medium salinity on cytoplasmic KCl content and proteome acidity in Halorhodospira. a, dependence on medium salinity of cytoplasmic K⁺ (open symbols) and Cl⁻ (closed symbols) content of H. halophila (circles and solid lines), H. halochloris (squares and dashed lines), and H. salinarum (triangles and dotted lines) as determined by plasma emission spectrometry and calorimetrically, respectively. The typical K⁺ and Cl⁻ contents of E. coli near 200 Mw are indicated by the arrow. b, elemental composition of H. halophila grown in media containing 5% (black), 15% (red), and 35% (blue) NaCl compared with that of E. coli (green). a.u., arbitrary units. c, effect of medium NaCl concentration (5, 15, and 35%) on proteome acidity of H. halophila as detected by isoelectric focusing gel electrophoresis.
Proteome Acidity and $K^+$ Accumulation in Halophiles

Proteins in a highly saline cytoplasm are likely to face two main risks: precipitation by salting out and effective dehydration of the protein surface, resulting in loss of function. The function of the negatively charged protein surface may be to provide electrostatic repulsion to prevent such aggregation (27). However, both an acidic proteome and an alkaline proteome would be expected to achieve this goal. With respect to water solubility, Glu and Asp are favored over Lys and Arg, and this may contribute to the proteome acidity in halophiles (28, 29). Regarding the risk of protein dehydration, it has been noted that ionized Glu and Asp side chains are intrinsically highly hydrated, whereas Lys and Arg side chains are weakly hydrated (28, 30). Therefore, abundant Glu and Asp side chains on the protein surface can effectively compete with $K^+$ for water molecules and thus recruit the hydration shell needed (31) for protein function. This argument can explain the observation that all halophilic proteins exhibit a strong preference for Glu/Asp-$K^+$ interactions at the exclusion of the Lys/Arg-$Cl^-$ interactions and is consistent with the analysis of the surface of obligately halophilic proteins as observed in crystal structures (16, 32, 33). It is likely that protein surface hydration and protein solubility are strongly related; recent results indicate that negative charges on the protein surface increase protein solubility (34). The analysis provided here implies that adequate protein surface hydration (resulting both in an adequate hydration shell and sufficient solubility) necessitates the acidic character of proteins in halophiles that use $K^+$ as a main osmoprotectant.

Adaptive Neutral Evolution as the Cause of Extreme Proteome Acidity—The hydrating effect of an excess of Glu and Asp on the protein surface provides an explanation for the acidic nature of proteins from extreme halophiles. However, it leaves both the adaptive value and the molecular mechanism of the salt requirement for the folding and function of many of these proteins unexplained. We apply recent insights on the molecular evolution of proteins to understand this issue.

The majority of random point mutations in proteins result in a mild destabilization, which can subsequently be resolved by compensating stabilizing substitutions elsewhere in the protein (35). In the case of proteins that function for prolonged periods of time in a highly saline cytoplasm, a situation can be envisioned in which random mutations will tend to disrupt stabilizing interactions as they are present in initially $K^+$-compatible but non-halophilic proteins. Subsequent mutations that recover protein stability now occur in the protein present in a highly saline cytoplasm and can therefore recruit weakly stabilizing interactions between the already negatively charged protein surface and $K^+$ ions through the introduction of additional Glu and Asp residues. Such cation-binding sites have indeed been observed both in biophysical experiments (13) and in crystal structures of obligately halophilic proteins (16, 32, 33). These weak $K^+$-binding sites resemble the interactions that cause protein denaturation at molar concentrations of urea (36), except that they stabilize the native conformation.

This evolutionary ratchet would lead to a progressive increase in proteome acidity and a dependence of protein stability on salt. Given sufficient time, this process will result in the partial replacement of non-halophilic stabilizing interactions by halophilic stabilizing interactions between the negatively

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**FIGURE 5. Effect of cell washing procedure on elemental analysis of H. halophila.** The results of x-ray microanalysis of $H. halophila$ cells obtained after washing the indicated cell samples with isotonic NaCl (a) and ammonium sulfate (b) solutions are depicted. a.u., arbitrary units.
charged protein surface and K⁺ ions. In this analysis, obligate protein halophilicity does not confer a selective advantage but develops as a result of genetic drift similar to the process of constructive neutral evolution (37), in which non-halophilic stabilizing interactions are progressively replaced by halophilic ones.

A comparison of the properties of *H. halophila* and *S. salinarum* provides a test case for this proposal. This test is based on the prediction that, on an evolutionary time scale, both proteome acidity and obligate protein halophilicity will become more pronounced as the evolutionary ratchet described above remains active. Because the entire Halobacteria class consists of extreme halophiles, the proteome of *H. salinarum* has evolved in a cytoplasm that has remained highly saline for prolonged periods of time. In contrast, proteome acidity appears to be rare among the Proteobacteria, and the acidity of the proteomes of *H. halophila* and *H. halocloris* is strikingly different. This indicates that proteome acidity in *H. halophila* is a recent development. These considerations are matched by two experimental observations. First, the level of proteome acidity of the extremely halophilic halobacteria is more extreme that that observed for *H. halophila* (Fig. 2a). Second, obligate protein halophilicity is prevalent in proteins from *H. salinarum*, whereas this is not the case for *H. halophila*. Interestingly, recent studies on the properties of malate dehydrogenase and glutamate dehydrogenase from *S. ruber* have revealed a heterogeneous level of enzyme halophilicity in this organism (38–40), matching its level of proteome acidity intermediate to that of *H. salinarum* and *H. halophila*. This analysis implies that *S. ruber* has been dedicated to the extremely halophilic lifestyle for a sufficient period of time to reach an intermediate stage of obligate protein halophilicity.

The above considerations indicate that an acidic proteome combined with KCl as a main osmoprotectant offers an attractive osmoprotection strategy without requiring obligate protein halophilicity. This strategy may thus be more widespread than previously thought, as supported by its identification in a halophilic Proteobacterium here. The results on the osmoprotection strategy of *H. halophila* reported here thus offer novel insights and avenues for further research on both the evolution of halophilic adaptations and the biophysics of protein-solvent interactions.

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