Global Pro ein-Le el Responses of *Halobac eri m salinar m* NRC-1 o Prolonged Changes in E ernal Sodi m Chloride Concen ra ions

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Halobacterium salinarum *H. salinarum*

terium sp. NRC-1, recently amended *H. salinarum*

Ke ords:

Biological systems have evolved mechanisms to appropriately respond to environmental stresses that can damage proteins and DNA.[1] A very common stress situation is the change in external osmolarity due to extended periods of drought or rain. Members of the family *Halobacteriaceae* are particularly vulnerable to decreases in external salinity, as they need at least 1.0−1.5 M NaCl (~6 to 9% w/v) for growth.[2] To avoid lysis under low-osmolarity or dehydration under high-osmolarity growth conditions, halophilic archaea possess active changes in the molecular concentrations of the environment.[3]

Although halophilic archaea typically thrive in hypersaline *Halobacteriaceae* is *Halobacterium salinarum* NRC-1 (formerly *Halobacterium* sp. NRC-1, recently amended[8]) with the genome sequence published in 2000.[9] *H. salinarum* NRC-1 belongs to the genus *Halobacterium*, the type genus of the family *Halobacteriaceae*.[10] To balance external osmotic pressure, halophilic archaea typically generate high intracellular concentrations of inorganic cations (predominantly K^+). Recent studies have revealed that some halophilic archaea (*H. salinarum*), however, can also, or alternatively, accumulate compatible solutes, such as trimethylammonium compounds, to balance their internal osmotic pressure.[11] Previously, this and other studies have focused on the response of *H. salinarum* NRC-1 exposed to different environmental stress situations, including UV radiation,

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with the particular analysis of proteomic changes, have been investigated and their significance discussed.

Culture Conditions and Growth Studies. *H. salinarum* NRC-1 was a kind gift from Prof. Stan-Lotter. *H. salinarum* NRC-1 was cultivated under aerobic conditions with exposure to room light in 20 mL of ATCC 2185 media containing 4.3 M NaCl (optimum NaCl concentration) at 37 °C on a rocking platform (160 rpm) for 5 days. Subsequently, 100 μL of this culture was taken and transferred (in triplicate) into fresh ATCC 2185 media containing 2.6 M NaCl (low osmotic condition), 4.3 M NaCl (optimal growth condition), and 5.1 M NaCl (high osmotic condition), respectively. These starter cultures were incubated under identical conditions for up to 1 week. To adapt *H. salinarum* NRC-1 to these changed conditions, 100 μL of 2.6, 4.3, and 5.1 M NaCl cultures was inoculated in 20 mL of fresh media containing 2.6, 4.3, and 5.1 M NaCl, respectively.

±0.25 and 0.2 Da, respectively; Met(O), Cys-carboxyamidomethylation and iTRAQ-4-plex reagents on the N-terminus, Lys

The relative quantification of proteins identified with iTRAQ was achieved during analysis by estimating the abundance of the reporter ion peaks (m/z 115,116, and 117). Most of the identified proteins showed a down-regulation in expression in both 2.6 and 5.1 M NaCl (Figure 3). An overall reduction in protein expression was observed following growth in the altered NaCl concentrations. Following incubation at 2.6 M NaCl, 106 of the identified proteins were expressed at lower levels, compared to 62 of the proteins expressed at higher salt. A similar effect was observed following incubation at 5.1 M NaCl (66 proteins higher expressed compared to 75 proteins lower expressed).

General Proteome Changes Associated with Primary Metabolism. A key component of the archaeal and bacterial stress response is the down-regulation of genes that are not necessary for survival, while activating others whose function is to protect the cell.[28,29] In accordance with this premise, we found four flagellin proteins (FlaA2, FlaB1, FlaB2, and FlaB3)

Table 2.

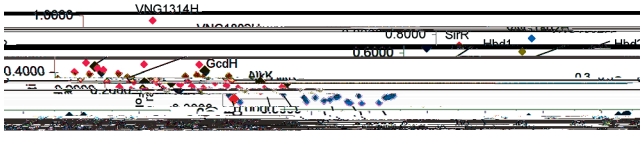

Fig re 5.

Fig re 6.

In addition, no changes in the proteins involved in the lipid-modifying pathway were identified during low- or high-osmotic growth. The only known mechanism for the biosynthesis of haloarchaeal polar lipids is via the mevalonate (MVA) pathway.[31,32]

VNG1339C (0.34 ± 0.19), and VNG1802H (0.75 ± 0.13). VNG1314H showed the highest increase and protein modeling revealed its structural similarity to flavodoxin 2 (pdb: 1YOB) from *Azotobacter vinelandi* (Supporting Information Table 3). Flavodoxins are small electron transfer proteins that contain one molecule of noncovalently but tightly bound flavin mononucleotide (FMN) as a redox active component[46] and are required for a variety of cellular functions, including the activation of the ribonucleotide reductase.[46,47] The ribonucleotide reductase of *H. salinarum* NRC-1 was also shown to be highly up-regulated under these low salt conditions. Structural predictions for the hypothetical protein VNG1802H did not result in any match with a known sequence or functional group.

Proteomic analysis of *H. salinarum* NRC-1 following different osmotic conditions with the iTRAQ-LC/MS systems showed a broad range of proteins involved in the response to prolonged osmotic stress. The strongest responses were recorded following exposure to 2.6 M NaCl, with a global down-regulation of the translational apparatus, up-regulation of chaperones and proteases, and changes in the metabolic activity. One of the most intriguing changes in the metabolic activity was the up-regulation of the bacteria-like fatty acid β-oxidation pathway, previously not known to be actively involved in the haloarchaeal energy cycle. Of specific interest was the large number of uncharacterized proteins that responded to changes in the external osmotic status, in particular protein VNG1802H. Further in-depth studies are necessary to elucidate their function and structural adaptation in greater detail. This and other studies[15,16,21] lay the foundation for further investigations into the behavior of halophilic archaea with regards to changes in external salt concentrations. This is of particular interest, as we are now observing halophilic archaea in environments where the NaCl concentrations are far below what was previously considered as optimal.[6,7,48]

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S ppor ing Informa ion A ailable: Tables of proteins differentially expressed following incubation at 2.6 and 5.1 M NaCl and hypothetical proteins identified in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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