Time Course Transcriptome Changes in *Shewanella algae* in Response to Salt Stress

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Abstract

*Shewanella algae*, which produces tetrodotoxin and exists in various seafoods, can cause human diseases, such as spondyloepidiscitis and bloody diarrhea. In the present study, we focused on the temporal, dynamic process in salt-stressed *S. algae* by monitoring the gene transcript levels at different time points after high salt exposure. Transcript changes in amino acid metabolism, carbohydrate metabolism, energy metabolism, membrane transport, regulatory functions, and cellular signaling were found to be important for the high salt response in *S. algae*. The most common strategies used by bacteria to survive and grow in high salt environments, such as Na+ efflux, K+ uptake, glutamate transport, and biosynthesis, and the accumulation of compatible solutes, were also observed in *S. algae*. In particular, genes involved in peptidoglycan biosynthesis and DNA repair were highly and steadily up-regulated, accompanied by rapid and instantaneous enhancement of the transcription of large- and small-ribosome subunits, which suggested that the structural changes in the cell wall and some stressful responses occurred in *S. algae*. Furthermore, the transcription of genes involved in the tricarboxylic acid (TCA) cycle and the glycolytic pathway was decreased, whereas the transcription of genes involved in anaerobic respiration was increased. These results, demonstrating the multi-pathway reactions of *S. algae* in response to salt stress, increase our understanding of the microbial stress response mechanisms.

Introduction

*Shewanella* belongs to the order Alteromonadales and the family Alteromonadaceae, the latter of which is a member of the gamma subdivision of Proteobacteria [1]. It is a Gram-negative bacterium that is capable of both aerobic and anaerobic respiration. More than 50 species of *Shewanella* have been recognized [2]. *Shewanella* is able to survive in a wide range of environments, including spoiled food and deep-sea and freshwater lake sediment as well as animals’ and patients’ blood and intestines [3–11]. Therefore, *Shewanella* has been suggested to be a good candidate model for studying how microorganisms respond to environmental stresses, such as osmolarity, temperature, and pH [12–16].

*Shewanella* has been detected in environments ranging from fresh water to hypersaline environments, and it has demonstrated its tolerance to a wide range of salt concentrations. Many *Shewanella* species are marine microorganisms and, therefore, are naturally tolerant to relatively high levels of salt. Common mechanisms that bacteria used to respond to high salinity include the exclusion of harmful ions via a variety of transport systems and the accumulation of compatible solutes through uptake or biosynthesis [17]. For *Shewanella*, a recent study of *S. oneidensis* in elevated salt conditions suggested that the down-regulation of flagellar-related genes might be necessary to conserve energy for sodium transport [18].

*S. algae*, a member of the *Shewanella* genus, can cause human diseases, such as spondyloepidiscitis and bloody diarrhea [19–21]; therefore, it has attracted much attention in microbiology. *S. algae* strains that produce tetrodotoxin and exist in various seafood are recently obtained from anal swabs of patients with food poisoning [22,23], and the strains are able to grow in high salt levels. The ability to adapt to high salt can be applied to their survival in seafood and other foods that contain high salt levels. In this study, we explored the responses and the possible adaptive mechanisms of *S. algae* strain to elevated salt stress by analyzing the transcriptome profiles of high salt cultures at different time points.

Materials and Methods

The Strain

*S. algae* strain 2736 (named MAS2736 previously) was one of strains isolated from the anal swab sample of a food poisoning patient with diarrhea and weak nerval symptom [22,23]. It can produce tetrodotoxin.

Growth Conditions

To identify the highest salt concentration that *S. algae* could tolerate, strain 2736 was cultivated for 5 h to an OD600 of 1.6 and was then used for seed cultures. The seed cultures were diluted to
1:100 and then cultivated in triplicate in LB broth (1% tryptone, 0.5% yeast extract) containing 0.5%, 3%, 6%, 8%, or 9% NaCl at 37°C and 200 rpm. The growth rates were measured spectrophotometrically (OD_{400}) once every hour in triplicate.

**Sample Preparation**

*S. algae* strain 2736 was cultivated in biological triplicate in LB broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl) for 3 h until it reached mid-log phase (OD_{600} = 1.6). Some of the cultures were fixed with a 2:1 volume of RNA protect Bacteria (Qiagen), harvested by centrifugation (5,500 rpm at 4°C for 10 min), and then were stored at −80°C. The time of culture harvest was defined as time zero. The remaining cultures were harvested by centrifugation (5,500 rpm at 4°C for 10 min) and were resuspended in PBS twice; then, they were cultivated in LB broth containing 8% NaCl. The samples were harvested at four time points (0, 1, 4, and 14 h) in biological triplicate.

**Total RNA Extraction**

Total RNA was isolated from the pellet using the RNeasy Mini kit (Qiagen). Genomic DNA was removed by incubation with DNase (Promega). RNA quality was determined using an Agilent 2100 Bioanalyzer. The extracted total RNA was sequenced.

**RNA Sequencing**

rRNA was removed using a kit (BGI Tech) after the total RNA was collected from the prokaryote. Fragmentation buffer was added to disrupt the mRNA into short fragments. Using these short fragments as templates, random hexamer primers were used to synthesize the first-strand cDNA. Second-strand cDNA was synthesized using buffer, dATPs, dGTPs, dCTPs, dUTPs, RNase H, and DNA polymerase I after removing the dNTPs. Short fragments were purified using the QiaQuick PCR extraction kit and were resolved in EB buffer; the ends were repaired, and a poly(A) tail was added. Then, the short fragments were connected to sequencing adapters. The UNG enzyme (BGI Tech) was used to degrade the second-strand cDNA, and the product was purified using the MiniElute PCR Purification Kit prior to PCR amplification. Finally, the library was sequenced using an Illumina HiSeq2000 system.

**RNA-Seq Analysis**

**Real-time Quantitative PCR (qRT-PCR)**

Superscript III first-strand synthesis system (Invitrogen) was used to generate cDNA using 1 μg of RNA and oligo dT primer, according to the manufacturer’s instructions. The qRT-PCR amplifications were performed using the SYBR Green EX Taq mix (TaKaRa) on a Bio-Rad CFX96 Real-Time PCR system. The relative expression level of the specific genes were determined by calculating 2^{-ΔΔCt} compared with the expression level of 0 h time point using 16s rRNA gene as an internal control. The qRT-PCR reactions were performed in triplicate for two biological replications. The specific primers for each gene are shown in Table S1.

Standard curves were generated for each gene to evaluate primer efficiency and for data analysis. *S. algae* strain 2736 was cultivated in biological triplicate in LB broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl) for 5 h (OD_{600} = 1.6) then total RNA was extracted, digested and converted to cDNA. Using 10-fold serial dilutions of the cDNA templates, qRT-PCR amplifications were performed. The standard curve was generated by plotting the Ct values versus ten times serial dilutions of the RNA templates ranging from 2.5 × 10^3–2.5 × 10^−4 ng/µl.

**Data Analysis**

In our analysis, the expression of each gene at 1 h, 4 h and 14 h was compared with that of 0 h, respectively. RPKM (Reads Per kb per Million reads) method [42] was used for the calculation of gene expression difference. False Discovery Rate (FDR) control is used in multiple hypothesis testing to correct for p-value [42]. The genes with FDR ≤0.001 and the ratio greater than 2 were defined as differentially expressed genes (DEGs) compared with 0 h. These DEGs were then used for GO term functional analysis and KEGG pathway analysis.

**Results and Discussion**

**Global Transcriptional Changes of *S. algae* 2736 in Response to Salt Stress**

We harvested *S. algae* strain 2736 cells exposed to salt stress, and the gene transcript levels were serially monitored at 0, 1, 4, and 14 h. The overall transcriptional profiles revealed that a considerable subset of genes was involved in the response of *S. algae* 2736 to salt stress. To validate the transcriptome data, five open reading frames (ORFs) for the four time points were selected for real-time quantitative PCR analysis. The relative transcript levels were normalized to the levels of 16S rRNA. The results showed that the transcriptome data were highly correlated with the qRT-PCR data (R^2 = 0.946/0.976/0.934) (Fig. S1). Standard curves were generated for each gene to evaluate primer efficiency and for data

**Figure 1. Growth curves for *S. algae* 2736 grown in LB broth.** *S. algae* 2736 cells were cultured in LB broth containing 0.5%, 3%, 6%, 8%, or 9% NaCl. doi:10.1371/journal.pone.0096001.g001
showed initial up-regulation, but transcriptional levels were split into 19 clusters (Fig. 3). Clusters 1, 2, and 3 showed a process and signaling, and translation could play important roles in modulating the cellular activities that allowed S. algae 2736 cells to adapt to salt stress. In summary, S. algae 2736 cells displayed a wide range of transcriptional alterations after exposure to elevated salt conditions, suggesting that S. algae resisted high salt stress through multiple strategies.

Alterations of High Salt-related Genes in S. algae 2736

Sigma factor. In our study, four putative sigma factors were found to be regulated in S. algae 2736 after exposure to elevated NaCl conditions, including GL2494, GL2061, GL2286, and GL3474 (Fig. 4A). GL2494, or the RNA polymerase nonessential primary-like sigma factor, which has 75% homology with RpoS, showed significantly sustained up-regulation beginning 1 h after salt stress. RpoS is a global regulatory factor that regulates genes that are mostly related to stress resistance. In E. coli, RpoS positively regulates approximately 10% of the genes in the whole genome when the bacterium encounters acute environmental stress, such as hyperosmosis, nutrient deficiency, low pH, and heat shock [24,25]. GL2061 and GL0228 are RNA polymerase sigma-70 factors that belong to the extracytoplasmic function (ECF) subfamily. ECF sigma factors play key roles during various stress responses and morphological development [26]. GL2061 was immediately up-regulated after salt stress, and it remained at a high level, whereas GL0228 displayed relatively delayed up-regulation, starting 4 h after salt exposure. GL3474, the RNA polymerase sigma factor for flagellar operon FliA, was highly down-regulated at 14 h after salt stress. Our data indicate that sigma factors contributed to the regulation of salt-inducible genes in S. algae 2736.

Na⁺ efflux, K⁺ uptake, and glutamate accumulation. In E. coli, Na⁺ efflux commonly occurs upon exposure to high salt conditions, along with simultaneous activation of K⁺ uptake, thus resulting in high levels of K⁺ ions. Additionally, the cells accumulate glutamate to neutralize the large amounts of cation accumulation [27]. The primary response of S. algae 2736 to salt stress is similar to that of E. coli. As expected, two genes encoding Na⁺ efflux transporters and five genes encoding Na⁺/H⁺ antiporters were found to be highly up-regulated after 1 h of salt exposure (Fig. 4B). Bacterial cells have three diverse K⁺ transporter systems that maintain the desired concentration of internal K⁺: Kup, Trk, and Kdp [28,29]. In S. algae 2736, trkH, KdpA, and KdpB were significantly up-regulated at all three time points after salt stress (Fig. 4B), demonstrating their importance in the adaptation of S. algae to high salt. Additionally, genes encoding the aminobenzoyl-glutamate transporter showed dramatic and immediate up-regulation, especially after 1 h of salt stress. In contrast, the genes encoding the large and small chains of glutamate synthase, which are required for glutamate synthesis, displayed delayed up-regulation patterns and were up-regulated at 14 h after salt stress (Fig. 4B). These data indicate that, at an early stage, the importation of glutamate from the outside was the primary mechanism used to counter high salt stress in S. algae 2736. As a secondary response, glutamate synthesis was then activated, resulting in the continual adaption of S. algae to its high salinity environment. In contrast, we detected a dramatic and sustained increase in the transcription of genes encoding

![Figure 2. Statistical chart of S. algae 2736 DEGS in response to salt stress. Compared to the transcriptional level at 0 h, 710 genes and 507 genes were up-regulated and down-regulated, respectively. At 4 h, 835 genes and 227 genes were up-regulated and down-regulated, respectively, whereas 883 genes were up-regulated and 180 genes were down-regulated at 14 h.

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whereas choline played a later role. It is important compatible solute to protect against the high salt stress, as well as genes involved in glutamate and proline biosynthesis, were highly induced by NaCl stress in _S. algae_ 2736.

**Accumulation of compatible solutes.** The most common strategy used by bacteria to survive and grow in high salt environments is the accumulation of compatible solutes, either by uptake or by biosynthesis, including glycine betaine, choline, carnitine, and trehalose [31]. In _S. algae_ 2736, significant changes in compatible solutes were observed at the transcript level under high salt conditions (Fig. 4C). In particular, the expression of the glycine betaine/carnitine transporter was up-regulated at 1 h, 4 h, and 14 h after salt stress. The transcript level of the choline transporter was up-regulated beginning at 4 h after salt stress. Two putative genes for betaine synthesis, choline dehydrogenase (BetA) and glycine betaine aldehyde dehydrogenase (BetB), were significantly up-regulated at all three time points in the presence of high salt. These data indicate that _S. algae_ 2736 first used betaine as an important compatible solute to protect against the high salt stress, whereas choline played a later role.

**Large- and small-ribosome subunits.** In the present study, we found that the transcript levels of the large- and small-ribosome subunits were significantly enhanced at 1 h after salt stress. However, the levels of most of the large- and small-ribosome subunits were restored to their initial levels at 4 h after salt stress (Fig. 4D), suggesting that _S. algae_ entered an adaptive phase. Consistent with our results, a previous study suggested that the removal of a putative ribosome maturation factor conferred salt tolerance on _E. coli_ cells [32]. Moreover, the majority of the 70S ribosome was dissociated into subunits after the addition of high concentrations of NaCl, and the dissociated subunits began to reassociate into the 70S ribosome after 4 or 6 h of salt stress. It has been suggested that the ribosome mediates a novel stress response pathway [33]. Taken together, the large- and small-ribosome subunits appeared to play roles in the adaption of _S. algae_ to high salt stress.

**Energy metabolism.** It has been suggested that critical enzymes involved in both aerobic and anaerobic respiration were significantly up-regulated in salt-stressed bacterial cells [18,34–37]. Notably, in our study, the transcript levels of fumarate reductase and aconitate hydratase, two critical enzymes necessary for the tricarboxylic acid (TCA) cycle, were down-regulated in _S. algae_ 2736 cells after 1 h of salt stress, and these levels increased again to the 0 h level or further increased at 4 h and 14 h. Similarly, glyceraldehyde 3-phosphate dehydrogenase and alcohol dehydrogenase, key enzymes involved in the glycolytic pathway, were down-regulated at the transcriptional level at 1 h after salt stress and returned to the 0 h level after 4 h of salt stress. However, enzymes critical for anaerobic respiration, such as periplasmic nitrate reductase, sulfite reductase, and sulfate reductase, were significantly enhanced at the transcriptional level in _S. algae_ 2736 cells at the three time points after high salt exposure (Fig. 4E). Based on these findings together, we conclude that, during the initial stage after exposure to high salt concentrations, _S. algae_ 2736 appears to utilize anaerobic respiration for energy production, instead of the tricarboxylic acid (TCA) cycle and the glycolytic pathway.

**DNA repair system.** The overall transcriptome profiles demonstrated that genes involved in DNA base excision repair, mismatch repair, and homologous recombination were dramatically up-regulated in the salt-stressed _S. algae_ 2736 cells, especially at 1 h and 4 h after salt exposure (Fig. 4F). Three DNA helicases

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**Table 1. Time course distribution of up- and down-regulated genes within KEGG pathway.**

| KEGG class2 | Total | 1 h up(down) | 4 h up(down) | 14 h up(down) |
|-------------|-------|--------------|--------------|---------------|
| Amino acid metabolism | 93    | 51(17)       | 50(2)        | 33(3)         |
| Carbohydrate metabolism | 102   | 30(48)       | 36(14)       | 38(17)        |
| Energy metabolism | 69    | 32(20)       | 27(7)        | 40(6)         |
| Lipid metabolism | 28    | 10(2)        | 18(0)        | 16(4)         |
| Nucleotide metabolism | 32    | 14(6)        | 16(6)        | 9(8)          |
| Glycan biosynthesis and metabolism | 11 | 11(0) | 8(0) | 6(0) |
| Metabolism of cofactors and vitamins | 34 | 64(51) | 74(23) | 76(21) |
| Xenobiotics biodegradation and metabolism | 8 | 3(4) | 3(2) | 6(2) |
| Enzyme families | 43    | 15(11)       | 22(3)        | 29(3)         |
| Folding, sorting and degradation | 32 | 15(8) | 14(3) | 9(5) |
| Genetic Information processing | 68 | 28(21) | 31(7) | 29(6) |
| Cell growth and death | 6 | 2(1) | 2(0) | 5(0) |
| Membrane transport | 108 | 30(35) | 53(17) | 65(11) |
| Replication and repair | 51 | 26(8) | 35(5) | 20(0) |
| Signal transduction | 79 | 24(29) | 30(13) | 44(8) |
| Transcription | 66 | 32(19) | 30(12) | 29(8) |
| Cell motility | 14 | 4(2) | 7(0) | 9(0) |
| Cellular processes and signaling | 172 | 78(43) | 90(27) | 98(15) |
| Translation | 48 | 35(1) | 10(5) | 2(11) |
| Function unknown | 499 | 177(150) | 236(68) | 268(42) |
| NA CLASS | 230 | 78(68) | 99(28) | 106(29) |

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encoded by RuvABC were up-regulated at 1 h and 4 h after salt stress. RuvB might function at the Holliday junctions to overcome regions of DNA heterology and DNA lesions [38]. DNA helicases are known confer high salinity tolerance in tobacco [39]. These results indicate that salt stress might induce the impairment of nucleic acid synthesis, thereby triggering the corresponding repair systems.

**Peptidoglycan biosynthesis.** We observed that key genes related to peptidoglycan biosynthesis were up-regulated in *S. algae* 2736 cells by salt stress (Fig. 4H). It has been documented that several physical changes, such as dehydration and shrinkage of the cells, occur immediately in *E. coli* cells after osmotic shock caused by an increase in salt concentration, in response to the changes in environmental osmolarity [40,41]. Therefore, our data also indicate that peptidoglycan, the dominant component of the Gram-negative bacterial cell wall, serves as an osmoprotectant and, therefore, helps *S. algae* to combat the dehydration caused by high levels of salt.

**Flagellar system.** Among all of the flagellar-related genes, three genes were down-regulated, whereas another three genes were up-regulated at 1 h after salt exposure. Importantly, seven genes were up-regulated at 4 h after high salt exposure, and 14 genes were up-regulated at 14 h after salt administration. No genes were found to be down-regulated at either 4 h or 14 h after salt stress (Fig. 4G). Previous studies have documented in detail the transcriptional regulation of flagellar genes in bacterial species. For example, *S. oneidensis* MR-1 responded to elevated salt concentrations by down-regulating flagellar assembly genes, accompanied by a decrease in cell motility. A dynamic process was observed in *Desulfovibrio vulgaris* Hildenborough. During early time points during salt stress, many chemotaxis-related genes were found to be up-regulated; at later time points during salt stress, only a few such genes were overexpressed, and the expression of most genes

**Figure 3. Classification of differentially expressed genes based on the dynamics of the transcript changes during the time course.**

The number of genes included within each cluster is reported in blue between parentheses.

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Figure 4. Alterations in high salt-related genes in S. algae 2736. The clusters were created using TMEL. (A) Sigma factor. (B) Na+ efflux, K+ uptake, and glutamate accumulation. (C) Accumulation of compatible solutes. (D) Large- and small-ribosome subunits. (E) Energy metabolism. (F) DNA repair system. (G) Flagellar system. (H) Peptidoglycan biosynthesis. Specific colors represent the different regulation patterns. Gray, no change. Red, up-regulation. Green, down-regulation.

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remained unchanged [18,34–36]. Our results were not consistent with the findings above, and one possible explanation could be that the up-regulation of flagellar genes at 14 h might not be related to the response to high salt but was instead related to the growth state of the bacteria.

In conclusion, in this study, we explored the possible mechanisms of the adaptation of *S. algae* to high salt conditions by performing transcriptome profiling combined with cell growth analyses. A broad set of differentially expressed genes was observed. Globally, genome-wide transcriptional analyses demonstrated that transcript changes, especially changes in amino acid metabolism, carbohydrate metabolism, energy metabolism, membrane transport, regulatory functions, and cellular signaling, seemed to be important for *S. algae* to respond to salt stress.

Importantly, we elucidated a temporal, dynamic process in the salt-stressed *S. algae* strain 2736 by monitoring gene transcription levels at 0, 1, 4, and 14 h. Exposure of *S. algae* to high salt could induce two different, but related, responses, which we defined here as the initial and prolonged responses. Initially, in response to the high salt environment, the *S. algae* strain quickly recruited central factors, such as K⁺, glutamate, and betaine, mainly through import, as represented by the apparent up-regulation of certain transport genes (e.g., K⁺ transporters, aminobenzoyl-glutamate transporters, and glycine betaine/carnitine transporter) at 1 h. Na⁺ efflux, the accumulation of large—/small-ribosome subunits, and the induction of the DNA repair system appear to be other initial mechanisms of the response of *S. algae* to elevated salt stress. It is worth noting that *S. algae* can rely on anaerobic respiration but not the tricarboxylic acid (TCA) cycle or glycolytic pathway for energy production during the initial stage of high salt exposure. When *S. algae* was persistently exposed to high salt concentrations, as a prolonged response, corresponding biosynthesis systems were triggered to play vital roles, resulting in the adaption of *S. algae* to salt stress. For instance, the expression of choline transport protein was increased beginning at 4 h after salt stress. However, some prolonged regulated genes did not appear to be related to the
high salt response but were instead related to the growth state of the bacteria. For example, the expression of flagellar genes and glutamate synthase were increased beginning 14 h after high salt exposure (Fig. 5).

Taking all of our findings together, we present a temporal dynamic response pattern of S. algae strain 2736 after exposure to high salt conditions, and our results elucidate the mechanisms that Shewanella utilizes to survive and adapt to environmental stress.

Supporting Information

Figure S1 Correlation of real-time qRT-PCR and RNA sequencing analyses.

(TIF)

References

1. Diuok RB (2011) Genome-level homology and phylogeny of Shewanella (Gammaproteobacteria: tertesomasales: Shewanellaceae). BMC Genomics 12:237.
2. Verma P, Pandey PK, Gupta AK, Kim HJ, Baik KS, et al. (2011) Shewanella indica sp. nov., isolated from sediment of the Arabian Sea. Int J Syst Evol Microbiol 61:2058–2064.
3. Pagani L, Lang A, Vedovelli C, Molini O, Rimenti G, et al. (2005) Soft tissue infection and bacteremia caused by Shewanella putrefaciens. J Clin Microbiol 41:2240–2242.
4. Nak R, Saiika L, Choudhury G, Das PP (2011) Isolation of Shewanella algae from rectal swabs of patients with bloody diarrhoea. Indian J Med Microbiol 29:422–425.
5. Zong Z (2011) Nosocomial peripancreatic infection associated with Shewanella putrefaciens. J Med Microbiol 60:1387–1390.
6. Shrishrimal K (2011) Recurrent Ochrobactrum anthropi and Shewanella putrefaciens bloodstream infection complicating hemodialysis. Hemodial Int 16:113–115.
7. Basir N, Yong AM, Chong VH (2011) Shewanella putrefaciens, a rare cause of splenic abscess. J Microbiol Immunol Infect 45:151–153.
8. Myers CR, Nealon KH (1988) Bacterial manganese reduction and growth with manganese as the sole electron acceptor. Science 240:1319–1321.
9. Pinhasi J, Berman T (2005) Differential growth response of colony-forming alpha- and gamma-proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Elat. Appl Environ Microbiol 69:199–211.
10. Jiang H, Dong H, Yu B, Liu X, Li Y, et al. (2007) Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. Environ Microbiol 9:2603–2611.
11. Skerratt JH, Bowman JP, Nichols PD (2002) Shewanella oleovorans sp. nov., a marine species isolated from a temperate estuary which produces high levels of polysaccharidic fatty acids. Int J Syst Evol Microbiol 52:2101–2106.
12. Yamada M, Nakaseko K, Tamegai H, Kato C, Usami R, et al. (2000) Pressure transcriptional, physiological, and metabolite analyses of the responses of Dissimilatory arsenate and sulfate reduction in sediments of two hypersaline, arsenic-rich soda lakes: Mono and Searles Lakes, California. Appl Environ Microbiol 67:1551–1555.
13. Lebreve O, Vanudevan N, Thanasekaran K, Moletta R, Godon JJ (2006) Microbial diversity in hypersaline wastewater: the example of tarnures. Extremophiles 10:505–513.
14. Li S, Xiao X, Li J, Liao J, Wang F (2006) Identification of genes regulated by changing salinity in the deep-sea bacterium Shewanella sp. WP5 using RNA arbitrarily primed PCR. Extremophiles 10:97–104.
15. Kulp TR, Hoelh SE, Miller LG, Salitkov C, Murphy JN, et al. (2006) Dissimilatory arsenate and sulfate reduction in sediments of two hypersaline, arsenic-rich soda lakes: Mono and Searles Lakes, California. Appl Environ Microbiol 72:6314–6320.
16. Leblanc L, Lebourd C, Levie F, Hartke A, Auffray Y (2003) Comparison between NaCl tolerant response and acclimation to cold temperature in Shewanella putrefaciens. Curr Microbiol 46:157–162.
17. Roesser M, Muller V (2001) Osmoadaptation in bacteria and archaea: common principles and differences. Environ Microbiol 3:743–754.
18. Liu Y, Gao W, Wang Y, Wu L, Liu X, et al. (2005) Transcriptome analysis of Shewanella oneidensis MR-1 in response to elevated salt conditions. J Bacteriol 187:2501–2507.
19. Gao H, Yang ZK, Wu L, Thompson DK, Zhou J (2006) Global transcriptional analysis of the cold shock response of Shewanella oneidensis MR-1 and mutational analysis of its classical cold shock proteins. J Bacteriol 188:4560–4569.
20. Gao H, Wang Y, Liu X, Yan T, Wu L, et al. (2004) Global transcriptome analysis of the heat shock response of Shewanella oneidensis. J Bacteriol 186:7796–7803.
21. Gresser M, Mbaye D, Deramond H, Grados F, Eb F, et al. (2003) First case of human leptospirosis due to Shewanella algae. Int J Infect Dis 14 Suppl 3:e261–264.
22. Wang D, Wang Y, Huang H, Liu J, Xiao D, et al. (2013) Identification of tetrodoxin-producing Shewanella spp. from fishes of food poisoning patients and food samples. Gut Pathog 5:13.
23. Wang YL, Wang DC, Zhou SW, Zheng JX, Liu Y, et al. (2009) Isolation and characterization of Shewanella spp. from patients of food poisoning. Zhonghua Liu Xing Bing Xue Za Zhi 30:836–840.
24. Hengge R (2009) Proteolysis of sigmaS (RpoS) and the general stress response in Escherichia coli. Rev Microbiol 60:667–676.
25. Hengge-Aronis R (2002) Recent insights into the general stress response regulatory network in Escherichia coli. J Mol Microbiol Biotechnol 4:341–346.
26. Hughes KT, Mathee K (1998) The anti-sigma factors. Annu Rev Microbiol 52:231–260.
27. Seator RD, Hill C (2002) Bacterial osmoadaptation: the role of osmotolysates in bacterial stress and virulence. FEMS Microbiol Rev 26:49–71.
28. Schlosser A, Meldorf M, Stumpe S, Bakker EP, Epstein W (1995) TrkH and its homolog, TrkG, determine the specificity and kinetics of cation transport by the Trk system of Escherichia coli. J Bacteriol 177:1908–1910.
29. Epstein W (1992) Kdp, a bacterial P-type ATPase whose expression and activity are regulated by turgor pressure. Acta Physiol Scand Suppl 607:193–199.
30. Nandakumar R, Wakyama M, Nagano Y, Kawaiura T, Sakai K, et al. (1999) Overexpression of salt-tolerant glutaminase from Micrococcus luteus K-3 in Escherichia coli and its purification. Protein Expr Purif 15:155–161.
31. van der Heide T, Ploodman B (2000) Glycine betaine transport in Lactococcus lactis is osmotically regulated at the level of expression and translocation activity. J Bacteriol 182:203–206.
32. Hase Y, Tarasawa T, Muto A, Himeno H Impairment of ribosome maturation of function confers salt resistance on Escherichia coli cells. Plas Dis One 15:67547.
33. Hase Y, Yokoyama S, Muto A, Himeno H (2009) Removal of a ribosome small subunit-dependent GTPase confers salt resistance on Escherichia coli. RNA 15:1766–1774.
34. Yin J, Gao H (2011) Stress responses of shewanella. Int J Microbiol 2011:461329.
35. Mukhopadhyay A, He Z, Alm EJ, Arkin AP, Baidoo EE, et al. (2006) Salt stress in Desulfovibrio vulgaris Hildenborough: an integrated genomics approach. J Bacteriol 188:4069–4078.
36. He Z, Zhou A, Baidoo E, He Q, Joshiakma MP, et al. (2011) Global transcriptional, physiological, and metabolite analyses of the responses of Desulfovibrio vulgaris hildenborough to salt adaptation. Appl Environ Microbiol 76:1574–1586.
37. Himels AP, Berks BC (2002) Specificity of respiratory pathways involved in the reduction of sulfur compounds by Sulfonella enterica. Microbiology 148:3631–3638.
38. Kaplan DL, O’Donnell M (2004) Twin DNA pumps of a hexameric helicase provide power to simultaneously melt two duplexes. Mol Cell 15:455–465.
39. Sanan-Mishra N, Pham XH, Sopory SK, Tuteja N (2005) Pea DNA helicase 45 regulates gene expression and salt stress tolerance. Plant Physiol 139:1121–1129.
40. Dehne K, Hulka H, Ahlgrimm M, Kruger B, et al. (2005) Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 3:624–630.

Figure S2 The standard curves for each gene.

(TIF)

Table S1 qRT-PCR Primers.

(DOCX)

Table S2 The raw data and the complete list of up and down regulated genes.

(XLS)

Author Contributions

Conceived and designed the experiments: BK XF DW. Performed the experiments: XF. Analyzed the data: BK XF PD. Contributed reagents/materials/analysis tools: BK XF DW. Wrote the paper: BK XF XY DW.