Global transcriptional, physiological and metabolite analyses of *Desulfovibrio vulgaris* Hildenborough responses to salt adaptation

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ABSTRACT

The response of *Desulfovibrio vulgaris* Hildenborough to salt adaptation (long-term NaCl exposure) was examined by physiological, global transcriptional, and metabolite analyses. The growth of *D. vulgaris* was inhibited by high levels of NaCl, and the growth inhibition could be relieved by the addition of exogenous amino acids (e.g., glutamate, alanine, tryptophan) or yeast extract. Salt adaptation induced the expression of genes involved in amino acid biosynthesis and transport, electron transfer, hydrogen oxidation, and general stress responses (e.g., heat shock proteins, phage shock proteins, and oxidative stress response proteins). Genes involved in carbon metabolism, cell motility, and phage structures were repressed. Comparison of transcriptomic profiles of *D. vulgaris* responses to salt adaptation with those of salt shock (short-term NaCl exposure) showed some similarity as well as a significant difference. Metabolite assays showed that glutamate and alanine were accumulated under salt adaptation, suggesting that they may be used as osmoprotectants in *D. vulgaris*. A conceptual model is proposed to link the observed results to currently available knowledge for further understanding the mechanisms of *D. vulgaris* adaptation to elevated NaCl.

INTRODUCTION

Desulfovibrio vulgaris Hildenborough is a sulfate-reducing bacterium (SRB) (Postgate, 1984) that can be found ubiquitously in anaerobic environments with sulfate (Ouattara and Jacq, 1992), such as gas pipelines, subsurface metal tanks, sediments, and off-shore oil production facilities (Blessing et al., 2001; Ye et al, 2004). These niches can also contain high concentrations of salt (e.g. NaCl). The potential of *D. vulgaris* for bioremediation because of its capacity to immobilize soluble forms of toxic metals as well as its deleterious role in metal corrosion have been widely recognized (Lovley and Phillips, 1994; Valls and Lorenzo 2002). Therefore, an understanding of mechanisms that *D. vulgaris* uses to survive and adapt to environments with high concentrations of salt (e.g. NaCl) may contribute to the development of successful bioremediation strategies and to the prediction of biocorrosion for petroleum energy-related industries.

The response of a microorganism to high salinity can be divided into two phases. The initial reaction to a sudden increase in salt concentration is termed "salt shock", while the subsequent survival and adaptation to a high salinity environment termed "salt adaptation". In both cases, an exposure of *D. vulgaris* to high salinity may present two different, but related environmental stimuli: one is osmotic stress, and the other is ionic stress (Han et al., 2005). Hyper-osmotic stress triggers water efflux from the cell that results in a reduction of turgor pressure and dehydration of the cytoplasm thereby increasing the ion concentration in the cytosol. Whereas ionic stress causes ions (e.g. Na^+) to enter the cytoplasm, leading to a further increase in the ion concentration, and subsequently damaging the membrane systems and deactivating key enzymes (Sleator and Hill, 2002).

Bacteria use a range of mechanisms to respond to high salinity. The most common is an accumulation of compatible solutes, such as glutamate (McLaggan et al., 1994; Botsford et al., 1994), trehalose (Strom and Kaasen, 1993), proline (Whatmore et al., 1990), glycine betaine (Ko et al., 1994), and ectoine (Jebbar et al., 2005). These solutes increase the internal osmotic pressure without interfering with vital cellular protein functions (Csonka and Hanson, 1996) and are accumulated either by biosynthesis or import. Another strategy used by bacteria is the exclusion of harmful ions (e.g. Na^+) via a variety of transport systems, such as Na^+/H^+ antiporters, Ca^{2+}/Na^+ exchangers, and Na^+/solute symporters (Ma et al., 1995; Padan and Krulwick, 2000; Liu et al., 2005), which may be present constitutively or induced in the presence of high salinity. Na^+/H^+ antiporters also play key roles for salt tolerance in *Cyanobacteria* (Waditee et al., 2002) and *Arabidopsis* (Shi et al., 2003). Bacterial responses to salt stress also include the induction of chaperons, chaperon-like proteins, and peptidases to correct errors caused by high salinity during DNA and protein synthesis (Hecker and Völker, 2001).

Genome-wide transcriptional analyses of salt and/or osmotic stress responses have been performed in many different bacteria and archaea, including *E. coli* (Weber and Koide, 2002), *B. subtilis* (Steil et al., 2003), *Shewanella oneidensis* MR-1 (Liu et al., 2005), *Yersinia pestis* (Han et al., 2005), *Pseudomonas aeruginosa* (Aspedon et al., 2006), *Sinorhizobium meliloti* (Domingguez-Ferreras et al., 2006), and *Methanosarcina mazei* (Pflüger et al., 2007). A wide set of differentially expressed genes were observed in all studies. Since the annotated *D. vulgaris* genome is available (Heidelberg et al., 2004), a series of transcriptional studies of *D. vulgaris* responses to different environmental stresses have been conducted (He et al., 2006; Clark et al., 2006; Chhabra et al., 2006; Bender et al., 2007; Mukhopadhyay et al., 2007). In particular, a study of the response of *D. vulgaris* to salt shock (Mukhopadhyay et al., 2006) demonstrated that

import of osmoprotectants (e.g. glycine betaine, ectoine) was the primary mechanism to counter hyperionic stress (Mukhopadhyay et al., 2006). This study also showed that several efflux systems, ATP synthesis pathways, and chemotaxis genes were highly up-regulated while flagellar biosynthesis and lactate uptake and transport systems were down-regulated (Mukhopadhyay et al., 2006). In other organisms such as *B. subtilis*, both salt shock and salt adaptation have been studied. Only a small number of genes were found to be differentially expressed in both treatments (Steil et al., 2003). This may be true for most mesophilic bacteria including *D. vulgaris*.

In this study, possible mechanisms of *D. vulgaris* adaptation to high salinity were explored via transcriptomic profiling combined with cell growth, physiological, and metabolite analyses. Here, salt adaptation is defined as that they have survived an initial salt stress and grown until the mid-lag phase even with a slow growth rate since an inoculation of *D. vulgaris* cells into a NaCl-added medium, while salt shock as that the mid-log growing *D. vulgaris* cells are exposed to NaCl for a short time (30-480 min). Based on this work definition, our objectives of this study were to: (i) examine transcript changes in amino acid metabolism and transport and energy metabolism in *D. vulgaris* in response to salt adaptation, (ii) identify potential osmoprotectants in *D. vulgaris* during salt adaptation, (iii) compare the global transcriptomic profiling of *D. vulgaris* in response to salt adaptation and salt shock, and (iv) elucidation of physiological states for initial and prolonged responses to stress. A conceptual model is proposed for adaptation to high salinity in *D. vulgaris*.

METHODS AND MATERIALS

Oligonucleotide probe design and microarray construction

The 70mer oligonucleotide microarray for *D. vulgaris* was constructed as described previously (He et al., 2006). All designed oligonucleotides were commercially synthesized without modification by MWG Biotech Inc. (High Point, NC). The concentration of oligonucleotides was adjusted to 100 pmol/µl. Oligonucleotide probes prepared in 50% DMSO (Sigma Chemical Co., MO) were spotted onto UltraGAPS glass slides (Corning Life Science, NY) using a Microgrid II Arrayer (Genomic Solutions Inc., MI). Each oligonucleotide probe had two replicates on a single slide. Additionally, 6 different concentrations (5 to 300 ng/µl) of genomic DNA were also spotted (4 replicates on a single slide) as general positive controls. In total, there were 7284 spots on the array. After printing, the oligonucleotide probes were fixed onto the slides by UV cross-linking (600 mJ of energy) according to the protocol of the manufacturer (Corning Life Science, NY).

Cell growth

D. vulgaris cultures were routinely grown in a defined lactate sulfate medium (LS4D). LS4D consisted of 60 mM sodium lactate, 50 mM Na_2SO_4, 8.0 mM $MgCl_2$, 20 mM NH_4Cl, 2.2 mM K_2HPO_4, 0.6 mM $CaCl_2$, 30 mM PIPES [piperazine-N,N-bis(2-ethanesulfonic acid)], 12.5 ml trace mineral solution per liter (Brandis and Thauer, 1981), NaOH to a pH of 7.2, and 1.0 ml of a 10 x vitamin solution per liter (Brandis and Thauer, 1981) added after autoclaving. As the reductant for the LS4D medium, 5 ml per liter of an anaerobic titanium citrate solution was used. This solution contained 20% (wt/vol) titanium(III) chloride, 0.2 M sodium citrate, and 8.0% (wt/vol) sodium carbonate. Cell growth was monitored at an optical density (OD) of 600 nm. For transcript analysis, the control and NaCl treatment samples were harvested at the mid-log phase (OD_{600} ~ 0.25), approximately 15 and 100 hrs after inoculation, respectively in triplicates at room temperature under anaerobic conditions, snap frozen in liquid nitrogen, and then stored at -

80°C. To determine the effects of amino acids on *D. vulgaris* growth, 100µl of 200 mM (the final concentration of 2 mM) amino acids that were prepared and filter-sterilized under anaerobic conditions were added into the LS4D medium with or without 250 mM NaCl. Cultures of 10 ml were incubated at 37°C in 30 ml anaerobic culture tubes and closed with butyl rubber stoppers and aluminum seals. For metabolite analysis, the biomass of *D. vulgaris* was cultured at 37°C in the LS4D minimal medium with or without 250 mM NaCl, and cells were collected at the mid-log phase (OD_{600} ~ 0.25), approximately 15 and 100 hrs after inoculation, respectively in triplicates at room temperature under anaerobic conditions, snap frozen in liquid nitrogen, and then stored at -80°C.

RNA extraction, purification and labeling

Total cellular RNA was isolated using TRIzol™ Reagent (Invitrogen Life Technologies, Carlsbad, CA). RNA samples were treated with RNase-free DNase I (Ambion, Austin, TX) and purified using the Mini RNeasy Kit (Qiagen, Chatsworth, CA). 10 µg of total cellular RNA was incubated at 70°C for 10 min in the presence of 10 µg of random primers (Invitrogen Life Technologies). The labeling reaction was catalyzed by 200 U of Superscript™ II RNase H⁻ reverse transcriptase (Invitrogen Life Technologies) in the presence of 500 µM dATP, dGTP, and dCTP, 25 µM dTTP, and 25 µM of the fluorophor Cy5-dUTP (Amersham BioSciences, UK). The reverse transcription reaction was allowed to proceed for 2 h at 42°C, followed by RNA hydrolysis in 1 N NaOH at 37°C for 10 min. The labeled cDNA probes were purified immediately using a QIAquick PCR purification column and concentrated in a Savant Speedvac centrifuge (Savant Instruments Inc., Holbrock, NY). For each biological sample, three slides were used. In addition to the duplicates of arrays on the same slide, three biological cell samples produced a total of 18 possible spots for each gene.

Genomic DNA extraction, purification and labeling

Genomic DNA was isolated and purified from *D. vulgaris* as described (Zhou et al., 1995). The purified genomic DNA was fluorescently labeled by random priming using Klenow fragment of DNA polymerase as described previously (He et al., 2005). The labeled genomic DNA was purified immediately using a QIAquick PCR purification column and concentrated in a Savant speedvac centrifuge (Savant Instruments Inc., Holbrock, NY).

Microarray hybridization, washing and scanning

Labeled genomic DNA (Cy3) was used as a common reference to co-hybridize with labeled RNA (Cy5) samples for each slide. Hybridization was performed by a TECAN HS4800Pro Hybridization Station (TECAN U.S., Durham, NC) based on the protocol of the manufacturer. This fully automated system allows hybridization, washing, and drying of up to 12 arrays for each extension (with a total of 4 extensions) at one time and provides more sensitive and consistent hybridization results. After hybridization, slides were scanned using a ProScanArray^{TM} microarray analysis system (Perkin Elmer®, Boston, MA).

Microarray data analysis

To determine fluorescent signal intensities for each spot, 16-bit TIFF scanned images were analyzed by using the software ImaGene version 6.0 (Biodiscovery Inc., Los Angeles, CA) to quantify spot signal, spot quality, and background fluorescent intensities. Any flagged spots (e.g. empty spots and bad spots) were removed before normalization. Details of microarray data analysis were previously described in Mukhopadhyay et al. (2006).

Real-time PCR quantification

In order to validate microarray hybridization results, twelve genes were selected for further analysis with real-time PCR. A specific primer pair for each gene was designed with a product of

approximately 100 bp, and detailed information about the selected genes and their primers is listed in Table S1. Real-time PCR (50 µl) was performed using Thermo-fast 96 PCR plates (Bio-Rad Laboratories, Hercules, CA), which were sealed with iCycler IQ optical quality tapes (BioRad Laboratories) on an iCycler IQ thermocycler (Bio-Rad, Laboratories). Each measurement was performed in three replicates. A dilution series of positive control DNA was used in the same plates as calibration standards. Positive control DNA was generated by standard PCR amplifications, and the amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN Inc., Valencia, CA) according to the protocol of the manufacturer. The purified PCR fragments were visualized and the size was confirmed by agarose gel electrophoresis, quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA), and then serially diluted to generate calibration standards. Data analysis was carried out with iCycler software (BioRad Laboratories). Based on the standard curve, a cycle threshold (CT) value was converted to the copy number of the gene in a sample. A ratio of the copy number for the treatment to the control was calculated and compared with the ratio of gene expression for each gene.

Metabolite extraction

D. vulgaris cells were grown in LS4D medium with (NaCl-stressed) or without (control) 250 mM NaCl. Both control and NaCl-stressed *D. vulgaris* cells were collected in triplicate at the mid-log phase and then pooled. A final volume of 400 ml was drawn from each pool of biomass. The cells were then harvested by centrifugation at 11,000 x *g* for 10 min at 4 °C. Metabolites were extracted via a methanol/water/chloroform extraction procedure, after which solid phase extraction (Oasis HLB, Waters, MA, USA) was used for the removal of salts from the sample (Baidoo et al., 2008). All amino acids, with the exception of betaine, glutamate and serine, were quantified via isotopically labeled amino acid standards, which were purchased from Sigma-

Aldrich (MO, USA) and C/D/N Isotopes (Quebec, Canada). Betaine, glutamate and serine were quantified based on their percent recoveries from the solid phase extraction cartridge.

Capillary electrophoresis (CE) conditions

CE separation conditions were used as previously described in Baidoo et al. (2008). However, for the detection of betaine, glutamate and serine, the sample was introduced to the capillary at 50 mbar for 250 seconds.

Mass spectrometry (MS)

MS analysis was conducted on an Agilent 6210 TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA) and an Agilent 1100 series isocratic HPLC pump for sheath liquid delivery. CE and electrospray ionization (ESI) MS coupling was achieved using an orthogonal coaxial sheath-flow interface, and the Agilent CE system was interfaced to the Agilent 6210 TOF LC/MS via a G1603A Agilent CE-MS adapter kit and a G1607A Agilent CE-ESI-MS sprayer kit (Agilent Technologies, Santa Clara, CA, USA). Both the Agilent CE system and Agilent 6210 TOF LC/MS were controlled by the Chemstation software package (Agilent Technologies, Santa Clara, CA, USA). A contact closure between both instrument set-ups was established in order to trigger the MS into operation upon the initiation of a run cycle from Chemstation. The Agilent 6210 TOF LC/MS was initially calibrated using the ES tune mix (Agilent Technologies, Santa Clara, CA, USA) and internally calibrated, during runs, via reference masses from tetrabutylammonium acetate (Sigma-Aldrich, St. Louis, MO, USA) and tetraethylammonium acetate (Fluka, Seelze, Germany). Data acquisition and processing were carried out by the Agilent MassHunter Work Station Console software package. Grounding of the CE-ESI-MS sprayer ensured that a full 30 kV potential difference was applied across the length of the capillary for more efficient separation. An electrical contact at the outlet end was provided by the

sheath liquid (methanol and water, 50:50, v/v) at a flow rate of 8 μl/min. Nitrogen gas was used as both the nebulizing (8 psi) and drying (8 liter/min) gases to facilitate the production of gas-phase ions. A drying gas temperature of 200°C was used throughout.

RESULTS

Growth of *D. vulgaris* during salt adaptation

D. vulgaris cells were grown in the LS4D medium with NaCl at different concentrations (0 to 500 mM). Low NaCl concentrations up to 100 mM did not significantly affect cell growth monitored by OD_{600}. The maximal OD_{600} were adversely affected at 250 mM NaCl (Fig. 1A), under which the final OD_{600} of *D. vulgaris* cells was decreased by 50% (Fig. 1A). *D. vulgaris* could not grow in the LS4D medium with 500 mM NaCl (Fig. 1A). To determine the effects of yeast extract (YE) on the growth of *D. vulgaris* with high salt, cells were grown in the LS4D medium with or without 0.5% (wt/vol) YE. With 250 mM NaCl supplementation, the culture with YE had an OD_{600} of approximately 0.9 versus 0.60 for the culture without YE, and grew more rapidly, though the final OD_{600} was still notably lower than the control (Fig. 1B). The results indicated that YE was beneficial for *D. vulgaris* growth and that some of its components (e.g., amino acids) could mitigate growth defects during a long-term exposure of *D. vulgaris* to NaCl. Experiments were conducted to examine transcriptional, metabolic, and physiological responses of *D. vulgaris* to 250 mM NaCl.

Transcriptional analysis of *D. vulgaris* responses to salt adaptation

For assessing transcript changes, the Z score cutoff for significant change was set to |2.0|. With this criterion, among 2647 ORFs that were assigned to 20 functional categories based on the TIGR roles, 195 and 184 ORFs were significantly up-regulated or down-regulated, respectively,

in cultures of *D. vulgaris* grown for an extended time in medium supplemented with 250 mM NaCl (Fig. S1). More than one third (111 ORFs or 15.2%) of the genes altered in expression encode hypothetical, conserved hypothetical, and function-unknown proteins. The other most significantly changed (up- or down-regulated) gene categories included amino acid metabolism with 8 (10.3%) ORFs, protein synthesis and fate with 16 (6.3%) ORFs, DNA metabolism with 8 (9.6%) ORFs, cell envelope with 32 (13.0%) ORFs, cellular process with 22 (13.8%) ORFs, energy metabolism with 37 (14.2%) ORFs, mobile and extrachromosomal element functions with 39 (30.5%) ORFs, signal transduction and regulatory functions with 37 (14.2%) ORFs, and transport and binding proteins with 41 (16.5%) ORFs (Fig. S1). In this study, our analysis was focused on the genes involved in amino acid metabolism and transport, energy metabolism, and regulatory functions, and general stress responses.

(A) Amino acid metabolism and transport. DVU0375 encoding a putative Glu/Leu/Phe/Val dehydrogenase family protein was highly expressed when *D. vulgaris* was adapted to 250 mM NaCl (Table 1). This increase might reasonably be expected to result in an increase in biosynthesis of glutamate, leucine, phenylalanine, and/or valine. The gene *aroE* for shikimate 5-hydrogenase that produces shikimate, a precursor of aromatic amino acids, was also up-regulated (Table 1), as were *trpB-1* encoding the beta subunit of a tryptophan synthase and *tnaA* encoding a tryptophanase (Table 1). The *D. vulgaris* genome has a predicted five-gene operon (*DVU2981-2985*) for leucine biosynthesis, of which three genes, *DVU2983* (*leuD)*, *DVU2984*, and *DVU2985* (*leuB)* were significantly down-regulated under salt adaptation (Table 1). Two operons involved in amino acid transport had a trend of up-regulation under salt adaptation although all genes in the operon were not significantly increased in expression. One was glycine/betaine/L-proline ABC transporter system of three genes (*DVU2297-2299*) and the other

was a high-affinity branched-chain amino acid ABC transporter system with five genes (*DVU2740-2744*) (Table 1). In addition, two genes, *DVU0724* for a sodium/Ala symporter family protein and *DVU3297* (*mtr*) for a tryptophan-specific transport protein were over-expressed under salt adaptation (Table 1). The results were interpreted to suggest that some amino acids, such as glutamate, tryptophan, and leucine might play a role in *D. vulgaris* responses to a salt challenge and that the import of glycine-betaine into the cell from the environment could be one of the mechanisms for *D. vulgaris* osmoregulation.

(B) Energy metabolism. The transcript levels for all genes (DVU0531 to DVU0536) in the *hmc* operon encoding the high-molecular-weight cytochrome (HMC) increased under salt adaptation (Fig. 2). Consistent with the up-regulation of the *hmc* operon, the genes encoding a formate dehydrogenase (DVU0587-0588), periplasmic [Fe] hydrogenase (DVU1769-1770), hydrogenase expression/formation protein (DVU1919), and iron-repressed flavodoxin (DVU2680) were also over-expressed (Fig. 2). In contrast, the transcript levels for genes encoding ferritin (DVU1568), pyruvate ferredoxin oxidoreductase (DVU1569-1570, and DVU1946), pyruvate kinase (DVU2514), and two putative carbon starvation proteins (DVU0598 and DVU0599) decreased under salt adaptation (Fig. 2). In addition, three genes (*glcD*, *pta*, and *ackA*) in the same predicted operon, and two genes (*glpF* and *glpK*) in another operon were down-regulated under salt adaptation (Fig. 2). The results suggest that electron transport in NaCl-stressed cells was much more active than the control cells, and that the utilization of carbon intermediates, such as pyruvate, glycolate, and glycerol was slower under salt adaptation.

(C) Sensory and regulatory genes. Two-component signal (TCS) transduction systems are a mechanism that bacteria use to sense and respond to dynamic environments (Stock et al., 2000). In the *D. vulgaris* genome, 63 putative sensory histidine kinases (HK) and 66 response regulators

(RR) are predicted (http://microbesonline.org). Fourteen putative transcriptionally realted genes altered their expression under salt adaptation with twelve (DVU0030, *rrf2, hrcA,* DVU1643, DVU1760, *flrC*, DVU2423, *lysX*, DVU2819, *pspF*, *flrA*, and DVU3313) up-regulated and only two (*phoH* and DVU3131) down-regulated (Fig. 3). The *rrf2* was reported to regulate the expression of *hmc* operon with 6 genes (DVU0531-0536) (Keon et al., 1997), and indeed the transcript levels for all those genes increased under salt adaptation (Fig. 2). Similarly, *pspF* (Fig. 3) is predicted to activate the *psp* operon with 3 genes (DVU2986-2988), which were all over-expressed 5.5- to 11.4-fold under salt adaptation.

Among histidine kinases and regulators that are dual function, four (*rrf1*, *cckA*, DVU2577, and DVU2578) putative sensory/regulatory genes were up-regulated, and nine (DVU0258, *atoC*, DVU0680, DVU0722, DVU0743, DVU2677, DVU2931, *fexB*, and DVU3221) were down-regulated under salt adaptation (Fig. 3). Consistent with the expression of *rrf2* and the *hmc* operon, *rrf1* was also increased (Fig. 2, Fig. 3). It has been reported that the expression of DVU0258 and DVU0680 was significantly down-regulated at the stationary phase in formate-based medium (Zhang et al., 2006). The expression of these two genes was also decreased under salt adaptation (Fig. 3). In addition, the transcripts of *cheY-1* and *cheY-2* significantly changed under salt adaptation with *cheY-1* down-regulated and *cheY-2* up-regulated. The results indicate that the expression of regulatory genes significantly changed during *D. vulgaris* adaptation to NaCl, suggesting that as-yet unknown regulatory networks are involved in cell survival during exposure to saline conditions.

(D) Ion transport and general stress response. The predicted iron uptake and transport regulon has two putative operons predicted to be regulated by Fur (Rodionov et al., 2004). One contains two genes, *feoB* (DVU2571) and *feoA* (DVU2572), and the other contains another *feoA*

(DVU2574). All three genes were up-regulated under salt adaptation and shock conditions (Table 2). With either NaCl treatment, the transcript levels of two genes predicted to be a MarR family gene (DVU0525), and a drug resistance transporter gene (DVU0526) in the same operon also increased while the expression of another two-gene (DVU2305-2306) operon related to phosphate transport decreased (Table 2).

Most genes involved in protein synthesis (e.g. ribosomal proteins) were repressed in NaCl-stressed cells (GEO Number), consistent with slower growth for salt-stressed cultures. The expression of a two-gene operon related to heat shock (DVU2441-2442) was highly increased, and the expression of peptidase-related genes (DVU2568-2569) was down-regulated (Table 2). *pspA* and *pspC* predicted to encode phage shock proteins were highly induced (Table 2). In addition, the expression of all seven genes (DVU0198-204) was induced in a seven-gene operon possibly encoding bacteriophage functions. In another operon of three genes, the transcript levels of two genes (*rdl* and *perR*) significantly increased although the transcript for gene *rbr* was not significantly up-regulated ($Z = 1.09$) (Table 2). The results indicate that *D. vulgaris* also uses general strategies for survival under salt adaptation conditions.

Validation of microarray data by real-time quantitative RT-PCR

To verify the microarray data, 12 ORFs (Table S1) with a range of expression levels were randomly selected for real-time quantitative reverse-transcription PCR using the same RNA prepared for microarray hybridization. The results showed that the microarray data were highly correlated with the RT-PCR measurements with $r^2 = 0.96$ ($n = 12$) (Fig. S2). This result is also consistent with other studies, such as nitrite stress (He et al., 2006) and growth transitions (Clark et al., 2006) using the same microarray platform.

Accumulation of amino acids in salt-stressed *D. vulgaris* cells

To determine if changes at the transcript level for amino acid biosynthesis genes were reflected at the metabolite level as well, metabolite assays of both control and treated cells were conducted. Compared to the control, in NaCl-stressed cells, the levels of eight amino acids, Glu, His, Ser, Lys, Gln, Ala, Leu, and Thr, and Glycine betaine increased between 1.5- to 8-fold with an accumulation of Glu at 10.25 nmoles/mg and Ala at 9.89 nmoles/mg. The levels of seven amino acids, Pro, Asp, Gly, Ile, Met, Arg, and Val changed in a range of 80-139% in comparison with the control. Curiously, the levels of the three aromatic amino acids, Phe, Tyr, and Trp, decreased to 62%, 57%, and 55%, respectively. Asparagine was not detected in the control (Table 3). Based upon both gene expression data and metabolite measurements, the most accumulated amino acids, such as Glu and Ala may be used as osmoprotectants in *D. vulgaris* adaptation to NaCl.

Relief of salt inhibition of *D. vulgaris* growth by an external addition of amino acids

To examine the effects of amino acids on salt adaptation of *D. vulgaris*, Glu, Ala, Lys, Trp and Leu were added individually to the LS4D medium and cell growth was monitored spectrophotometrically (OD_{600}). Significant growth effects (advantageous or deleterious) were not observed by the supplementation of the medium with amino acids for the control (Fig. 4A). However, when 250 mM NaCl was also added to the medium, the addition of Glu, Ala, Leu, and Trp significantly relieved the deficiencies in terms of growth rate, and the final OD_{600} of *D. vulgaris* increased by approximately 35%. The order of growth stimulation was Glu > Ala > Leu=Trp, and no growth improvement was observed with the addition of Lys (Fig. 4B). To be consistent with this observation, some amino acid transporters (DVU0724, DVU2341, DVU2740-2744, and DVU3297) were up-regulated under salt adaptation (Table 1). We interpreted the results to mean that particular amino acids (e.g., Glu or Ala) could increase the

ability of *D. vulgaris* to grow at high salinity, which was consistent with the accumulation of Glu and Ala in *D. vulgaris* under salt adaptation conditions (Table 3).

Comparison of effects of salt shock and salt adaptation on *D. vulgaris* gene expression

Based on a Z-score cut-off of |2.0|, the changes in transcript levels of *D. vulgaris* genes in responses to salt shock (at 120 min) and salt adaptation showed significant differences and similarities as well (Fig. 5). For salt-shocked data, the 120-min time point was selected since the largest number of genes were differentially expressed at that time point (Mukhopadhyay et al., 2006). In salt-adapted cultures, 228 ORFs were up-regulated (Fig. 5-I, Table S2), and 161 ORFs (Fig. 5-II, Table S3) were down-regulated that did not change their gene expression significantly under salt shock. Similarly, 154 ORFs were up-regulated (Fig. 5-III, Table S4), and 99 ORFs were down-regulated (Fig. 5-IV, Table S5) under salt shock that did not change gene expression under salt adaptation. On the other hand, transcriptomic profiling between salt adaptation and salt shock showed some similarities with 72 ORFs increased (Fig. 5-V, Table S6), and 53 ORFs decreased in expression (Fig. 5-VI, Table S7) under both conditions. These genes differentially expressed under both treatments represented only 16.3% of the 767 genes detected in the two conditions combined.

A comparison of transcriptomic profiling for amino acid biosynthesis of *D. vulgaris* under salt adaptation and salt shock showed that the cell might develop complex response mechanisms. First, the genes predicted to be involved in the synthesis of Glu, Trp, and Leu were different in the two treatments. DVU0375, potentially encoding glutamate dehydrogenase, was up-regulated under salt adaptation, while *gltB-1* and DVU3291 were up-regulated under salt shock; *trpB-1*, *trpB-2* and *trpA* were all up-regulated under salt shock, but only *trpB-1* under salt adaptation; five genes in the predicted operon of Leu synthesis had a trend of up-regulation

under salt shock, but a trend of down-regulation under salt adaptation (Table 1). Similarly, amino acid transport genes differentially expressed under salt adaptation and salt shock. For example, DVU0724, DVU2341, and *mtr* were all induced under salt adaptation but not under salt shock (Table 1). The results indicate that *D. vulgaris* may use different strategies to accumulate osmoprotectants to crop with osmotic stress under salt adaptation and salt shock.

Second, efflux systems including Na^+/H^+ antiporters, and cation/multidrug resistance proteins may function to pump cations (e.g., Na^+) out of the cell. Four genes (*acrB*, DVU2815, *acrA*, and DVU3327) were highly expressed under both salt adaptation and salt shock, but additional five genes (DVU0058, DVU0063, *mnhA*, DVU2816, and DVU3326) were only significantly up-regulated under salt shock (Fig. 6). To be consistent with more energy requirement for efflux processes, the F-type ATPase genes (DVU0774-0780) were observed to be up-regulated under salt shock but not under salt adaptation (Fig. 6). The results suggest that an exclusion of Na^+ from the cell might be a more important mechanism for an initial salt shock than for long-term salt adaptation in *D. vulgaris*.

Third, most of genes involved in carbon metabolism were down-regulated under both salt shock and salt adaptation. For example, all genes in the operon (DVU3025-3030) were generally down-regulated, and two putative carbon starvation protein A genes (DVU0598-0599) were highly expressed in *D. vulgaris* under both salt shock and salt adaptation (Fig. 2). Additionally, more genes including *ftn*, *porA*, *porB, oorB, glpF*, *glpK and DVU3349* were down-regulated under salt adaptation but did not significantly change their expression under salt shock (Fig. 2). The results suggest that the decreased carbon metabolism was consistent with slower growth during salt adaptation and possibly the re-direction of carbon flow to the production of osmoprotectants.

Fourth, two chemotaxis genes (*DVU0048* and *cheV-3*) were down-regulated under salt adaptation and one (*DVU1458*) for salt shock, and similarly, four methyl-accepting chemotaxis genes (*DVU0170*, *mcpD, DVU0608,* and *DVU2585*) were down-regulated under salt adaptation and one (*DVU0608*) for salt shock (Fig. 6). In addition, only one gene (*DVU1884)* was found to be up-regulated under salt adaptation and none for salt shock (Fig. 6). The results suggest that the cell trying to move away from stress conditions was not a major strategy under salt adaptation although it could be an initial response to salt shock. However, most genes involved in flagellar biosynthesis were not significantly changed under salt shock or salt adaptation with one gene (DVU3230) for salt adaptation and another gene (DVU3231) for salt shock down-regulated (Fig. 6), suggesting that the cell general motility may not be significantly changed under both salt adaptation and salt shock conditions.

Fifth, almost all significant changers of regulatory genes of *D. vulgaris* under salt shock could be also observed under salt adaptation, for example, *rrf2*, *DVU1645*, *pspF*, *flex*, and *cheY-2* (Fig. 3). However, more genes were only significantly down-regulated, or over-expressed under salt adaptation, and examples were *cckA*, *flrC*, *lysX*, *DVU3313*, and *cheY-1* (Fig. 3). The results indicate that *D. vulgaris* adaptation to elevated NaCl requires the coordination of more genes, and perhaps via a more complicated regulatory network as well.

Finally, *D. vulgaris* may also use common strategies to deal with salt adaptation and salt shock, which was reflected in the similar expression profiling of genes involved in amino acid synthesis and transport, such as *trpB-1,* the three-gene operon of glycine/betaine/L-proline ABC transporter, and the five-gene operon of high-affinity branched chain amino acid ABC transporter (Table 1), electron transfer, such as *hmcF-A* genes (Fig. 2), carbon metabolism, such as DVU0598, DVU0599, *por*, *glcD*, *pta*, and *ackA* (Fig. 2), inorganic ion transport, such as *feoA*,

feoB, and DVU2306 (Table 2), and general stress responses, such as DVU1729, DVU1730, *pspC*, *pspA*, *slyD*, and DVU0198-0200 (Table 2). The results suggest that those processes are critical for *D. vulgaris* to respond to both salt shock and salt adaptation.

DISCUSSION

In this study, we examined the adaptation of *D. vulgaris* to high salinity by transcriptomic, growth, and metabolite analyses. The global transcriptomic profiling identified groups of genes involved in amino acid biosynthesis and transport, energy metabolism, regulatory functions, and general stress responses that were important for salt adaptation. Metabolite measurements revealed that amino acids, such as glutamate might be used as osmoprotectants in *D. vulgaris*, and this notion was supported when an external addition of glutamate relieved salt inhibition. A comparison of transcriptomic profiling suggests that *D. vulgaris* may use different strategies to deal with the initial salt shock and salt adaptation. A conceptual model is constructed to provide an integrative understanding of the mechanisms of *D. vulgaris* adaptation to elevated NaCl.

One of the major strategies for bacteria to survive and grow under osmotic and salt stress conditions is to accumulate compatible solutes such as amino acids by import or synthesis. Our previous study (Mukhopadhyay et al., 2006) suggested that *D. vulgaris* might mainly import osmoprotectants (e.g., glycine betaine) to deal with salt shock, which may be an initial mechanism for salt adaptation. A few amino acid transport genes (e.g., *proW*, *livF*, *livM*, DVU2744) were highly up-regulated, and those high-affinity branched-chain amino acid ABC transporters are expected to import amino acids at low exogenousconcentrations . An accumulation of glutamate has been found to be an important osmoregulatory mechanism in bacteria, such as *Rhizobium meliloti* (Botsford and Lewis, 1990), *Salmonella typhimurium (*Botsford et al., 1994), *Erwinia chrysanthemi* strain 3937 (Goude et al., 2004), and *Escherichia*

coli (Csonka et al., 1996; McLaggan et al., 1994; Nandineni et al., 2004) in response to osmotic and/or salt stresses. In this study, metabolite assays showed that Glu was increased approximately 8-fold under salt adaptation compared to the control, and consistently, it was observed that an external addition of 2 mM Glu could significantly relieve salt growth inhibition in *D. vulgaris*. Glutamate is a central player in global nitrogen metabolism (Reitzer, 2003; Yan et al., 2007). In bacteria, it is well known that two pathways are responsible for glutamate synthesis: one is the GS-GOGAT cycle through the combined action of glutamine synthetase (GS, encoded by *glnA*) and glutamate synthase (GOGAT, GOGAT, encoded by *gltBD*), and the other is the GDH pathway by the action of glutamate dehydrogenase (GDH, encoded by *gdhA*). It appears that both pathways for glutamate biosynthesis exist in the *D. vulgaris* genome (http://www.microbesonline.org/). Although no genes have not been specifically assigned as glutamate dehydrogenase (GDH), two genes (DVU0375 and DVU0964) are annotated as Glu/Leu/Phe/Val dehydrogenase family proteins, and indeed, DVU0375 was significantly up-regulated under salt adaptation. Although no significant changes in expression were observed under salt adaptation, one gene (DVU1258) predicted to be glutamine synthetase (GS) and five genes (DVU1821-23, DVU2476, and DVU3291) for glutamate synthase (GOGAT) are present in the genome, and in contrast, there was a trend of over-expression of DVU1821 and DVU1823 (although the DVU1822 data is missing) in the same operon, and DVU3291 in another operon with other two genes under salt shock (Mukhopadhyay et al., 2006). However, it is not known if *D. vulgaris* mainly uses the GS-GOGAT pathway under salt shock, and the GDH pathway under salt adaptation for glutamate synthesis.

Although the intracellular concentration of Ala also reached 9.89 nmoles/mg, and one gene (DVU0724) predicted to encode a sodium/alanine symporter family protein was also up-

expressed, there have been no reports in the literature for this or the other accumulated amino acids (e.g., Leu, Ser, Gln, Lys) to be identified as osmoprotectants in bacteria. In growth assays, the addition of 2 mM Ala did alleviate the inhibitory effect of NaCl, which confirmed that the accumulation of Ala was indeed a response to salt adaptation. Of all the amino acids tested, only Lys did not appear to have a mitigating effect on the growth. However, the present data do not provide insight into the mechanism by which the accumulation or addition of these amino acids can alleviate salt inhibition, and further investigations are required to understand such mechanisms. This study indicates that *D. vulgaris* may accumulate compatible solutes, such as glutamate as an important strategy for survival and then growth under salt adaptation.

The transcriptomic data suggest a decrease in carbon metabolism under salt adaptation, and this was mainly reflected in the slower growth rate and in the down-regulation of carbon utilization genes and putative carbon starvation genes. First, although the membrane-bound lactate dehydrogenase (DVU0600, Z = -1.2) and the primary pyruvate:ferredoxin oxidoreductase (DVU3025, Z=-1.5) were not significantly altered, several genes (*porA*, *porB*, *oorB*, and DVU3349) that encoded pyruvate ferredoxin oxidoreductase were significantly decreased in expression under salt adaptation. Second, the genes *pta* and *ackA* were also significantly down-expressed, which may result in a decreaseed substrate-level ATP synthesis coupled to the conversion of acetyl-CoA to CoASH and acetate. In addition, two genes encoding putative carbon starvation proteins were also significantly down-regulated, suggesting that *D. vulgaris* utilized less carbon sources under salt adaptation, which is consistent with slow cell growth.

However, electron transport processes appeared to be more active or unchanged, which is supported by the expression of genes involved in both hydrogen cycling (Odom and Peck, 1981) and formate cycling (Heidelberg et al., 2004). In the proposed hydrogen cycling, periplasmic and

cytoplasmic hydrogenases are required to form a proton gradient for ATP synthesis and transport processes. In this study, a periplasmic hydrogenase (DVU1769-70) was up-expressed under salt adaptation although the transcript levels of genes encoding two cytoplasmic hydrogenases (Ech and Coo) were not significantly increased. Similarly, formate cycling also generates a proton gradient and provides energy for transport processes and ATP synthesis (Heidelberg et al., 2004). Indeed, two genes (DVU0587-8) encoding a formate dehydrogenase (Fdh) were up-regulated under salt adaptation in this study. Therefore, energy supply for transport processes could be kept stable or more active even though carbon metabolism activity in *D. vulgaris* was decreased in response to salt adaptation. Interestingly, sulfate reduction occurred in the cytoplasm remained unchanged although hydrogenase activity occurred in the periplasm seems to be increased. One possibility could be that such electron transport processes are modulated by the complicated c-type cytochrome network in *D. vulgaris* (Heidelberg et al., 2004).

In heterotrophic bacteria, such as *E. coli* and *B. subtilis*, the expression of salt-inducible genes was regulated by the sigma factors RpoS and SigB, respectively (Boor, 2006; Hengge-Aronis, 1996; Hecker et al., 1996; Hecker et al., 2001). In the photoautotrophic bacterium *Synechocystis sp.* PCC 6803, a few HKs were identified as sensors for salt stress (Marin et al., 2003). However, *D. vulgaris* appears not to have an RpoS ortholog, and no similar sensors, or regulators responsible for salt adaptation have been identified thus far. Fur is considered a global regulator in the *D. vulgaris* genome (Hemme et al., 2004). It was observed that high salinity caused iron deficiency and led to derepression of *fur* in *B. subtilis* (Hoffmann et al., 2002). Although the expression of *fur* did not significantly change under salt stress conditions, some genes predicted to be regulated by Fur, such as *DVU0273*, *DVU2574* (*feoA*), *DVU2680*, and *DVU3330* (Rodionov et al., 2004) did show increases in expression under salt adaptation (GEO

number). Similar results were also observed in *D. vulgaris* under nitrite stress (He et al., 2006) and salt shock (Mukhopadhyay et al., 2006). Thus, Fur may also play an important role in *D. vulgaris* under salt adaptation. The homolog of *fur*, *perR* (*DVU3095*) was significantly up-regulated under salt stress conditions. PerR is predicted to regulate oxidative stress genes and also responds to iron concentrations (Bsat et al., 1998; Chen et al., 1995). *rdl* that encodes rubredoxin-like protein and is predicted to be regulated by PerR was over-expressed under salt adaptation. However, since the function of most regulatory genes is unknown, an identification of regulatory networks in *D. vulgaris* as well as in other organisms in response to salt adaptation remains a great challenge.

Responses of *D. vulgaris* to elevated NaCl may follow a temporal pattern from salt shock to salt adaptation. This was first reflected in the changes in the expression of specific genes involved the import of osmoprotectants, efflux of harmful ions, energy supply, and cell motility. At an initial stage, *D. vulgaris* may mainly import efficient osmoprotectants, exclude Na^+ from the cell, and move away from the stressful environment. The dynamic change in the expression of chemotaxis genes was a good example. At early time points of salt shock, many chemotaxis-related genes were identified to be up-regulated; at later time points of salt shock, only a few such genes were over-expressed, and most of them remain unchanged (Mukhopadhyay et al., 2006). When *D. vulgaris* was in the process of adaptation to evaluated NaCl, only two chemotaxis genes were up-regulated, and most of them were down-regulated. Second, such a dynamic progress was seen in the accumulation of Glu. For example, the concentration of Glu was measured at a more than 8-fold increase under salt adaptation, but only about 2-fold increase under salt shock (Mukhopadhyay et al., 2006) in comparison with the control. In addition, the expression of regulatory genes also showed a similar trend, and more genes were observed to be

changed under salt adaptation than under salt shock. However, the mechanisms of dynamic adaptation to salt stress are unknown, which points to our on-going study on long-term evolution of *D. vulgaris* in response to evaluated NaCl.

Considering all together, a simple conceptual model is proposed for *D. vulgaris* adaptation to elevated NaCl (Fig. 7). Salt stress signal may be sensed by unknown HKs and then transduced to regulators, which regulate cellular activities of *D. vulgaris* in response to high salinity. In this model, only three categories of cellular activities (energy metabolism, general stress response, and amino acid or solute metabolism and transport) are emphasized based on our experimental results from growth, physiological, transcriptomic, and metabolite analyses. First, the transport of iron, phosphate (P_i), and amino acids into the cell and the exclusion of Na^+ from the cell require ATP hydrolysis. Although the transcriptomic data suggest a reduction of the ATP generation from the oxidation of lactate and pyruvate under salt adaptation, the over-expression of hydrogen oxidation and electron transport genes suggests that ATP generated from proton gradient may be increased. Perhaps consequently, sulfate reduction of *D. vulgaris* remained unchanged under salt stress conditions (Fig. 7A). Second, the most significant responses of *D. vulgaris* to salt adaptation were the decreased cell growth, and the over-expression of genes related to heat shock, phage shock, and oxidation. The latter is important for *D. vulgaris* to maintain the integrity of protein and membrane structures, prevent oxidative stress, suppress viral infections, and then adapt to such salt stress conditions (Fig. 7B). Third, the accumulation of small molecules (e.g., amino acids) to counter osmotic stress is one of the most important mechanisms in *D. vulgaris*. Our data strongly suggests that biosynthesis or import of one or more particular amino acids is the primary mechanism for salt adaptation. Of these, glutamate may be the most effective amino acid for *D. vulgaris* adaptation to a high salinity environment (Fig. 7C).

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Table 1 Expression changes of genes in *D. vulgaris* involved in amino acid biosynthesis and transport.

Locus	Gene	Predicted function	Salt adaptation		Salt shock	
A. Amino acid metabolism			Log_2R[a]	Z[b]	Log_2R	Z
DVU0085	*trpB-1*	Tryptophan synthase, beta subunit	2.48	**3.72**	1.44	**2.74**
DVU0470	*trpB-2*	Tryptophan synthase, beta subunit	-0.01	-0.01	1.16	**2.17**
DVU0471	*trpA*	Tryptophan synthase, alpha subunit	0.09	0.15	1.41	**2.73**
DVU2204	*tnaA*	Tryptophanase	1.81	**3.22**	0.27	0.47
DVU0115	*aroE*	Shikimate 5-dehydrogenase	1.58	**2.01**	0.74	1.19
DVU0375	NA[c]	Glu/Leu/Phe/Val dehydrogenase family protein	2.70	**3.69**	0.70	1.12
DVU1823	*gltB-1*	Glutamate synthase, iron-sulfur clustering-binding subunit, putative	0.20	0.16	1.35	**2.37**
DVU3291	NA	Glutamate synthase, iron-sulfur clustering-binding subunit, putative	0.30	0.48	1.54	**2.31**
DVU2981	*leuA*	2-isopropylmalate synthase	-0.18	-0.18	1.36	**2.51**
DVU2982	*leuC*	3-isopropylmalate dehydratase, large subunit, putative	-1.62	-1.67	1.23	1.94
DVU2983	*leuD*	3-isopropylmalate dehydratase, small subunit	-2.37	**-2.23**	1.01	1.86
DVU2984	NA	conserved hypothetical protein	-3.14	**-3.46**	0.98	1.87
DVU2985	*leuB*	3-isopropylmalate dehydrogenase	-1.54	**-2.25**	0.68	1.35
B. Amino acid transport						
DVU0724	NA	Sodium/Ala symporter family protein	1.42	**2.69**	0.53	1.04
DVU2297	*proW*	Glycine/betaine/L-proline ABC transporter, periplasmic-binding protein	1.59	**3.11**	1.62	**3.13**
DVU2298	*opuBB*	Glycine/betaine/L-proline ABC transporter, permease protein	0.82	1.42	1.43	**2.74**
DVU2299	*proV*	Glycine/betaine/L-proline ABC transporter, ATP binding protein	0.87	1.49	1.48	**2.56**
DVU2341	NA	Amino acid ABC transporter, permease protein, His/Glu/Gln/Arg/opine family	1.56	**2.76**	0.78	1.18
DVU2740	*livF*	High-affinity branched-chain amino acid ABC transporter, ATP-binding protein	3.18	**5.34**	0.61	0.96
DVU2741	*livG*	High-affinity branched chain amino acid ABC transporter, ATP-binding protein	0.54	0.64	1.05	1.66
DVU2742	*livM*	High-affinity branched chain amino acid ABC transporter, permease protein	1.79	**2.08**	0.93	1.76
DVU2743	*livH*	High-affinity branched-chain amino acid ABC transporter, permease protein	0.29	0.32	0.15	0.22
DVU2744	NA	High-affinity branched-chain amino acid ABC transporter, periplasmic amino acid binding protein	2.40	**4.48**	1.78	**3.20**
DVU3297	*mtr*	Tryptophan-specific transport protein	1.34	**2.48**	0.35	0.65

a: $\log_2$ (treatment/control); b: Z score; c: no gene name has been assigned to this locus yet.

Table 2 Expression changes of genes in representative operons of *D. vulgaris* under salt adaptation in comparison with salt shock.

Locus	Gene	Predicted function	Salt adaptation		Salt shock	
A. Inorganic ion transport and metabolism			**Log_2R[a]**	**Z[b]**	**Log_2R[a]**	**Z[b]**
DVU0525	NA[c]	Transcriptional regulator, MarR family	2.99	**2.76**	1.24	**2.30**
DVU0526	NA	Drug resistance transporter, putative	3.01	**5.05**	1.81	**3.39**
DVU2571	*feoB*	Ferrous iron transport protein B	2.87	**5.09**	1.88	**3.54**
DVU2572	*feoA*	Ferrous iron transport protein A, putative	2.11	**3.21**	1.98	**3.60**
DVU2574	*feoA*	Ferrous iron transporter component feoA	1.21	**2.13**	1.46	**2.31**
DVU2305	NA	Conserved hypothetical protein	-2.38	**-2.68**	-1.07	**-2.06**
DVU2306	NA	Phosphate transporter family protein	-1.55	**-2.89**	-1.02	-1.99
B. General stress response						
DVU1729	NA	Killer protein, putative	2.14	**2.13**	2.75	**4.76**
DVU1730	NA	DNA-binding protein	2.46	**3.36**	2.28	**3.91**
DVU2441	*hspC*	Heat shock protein, Hsp20 family	4.01	**7.42**	0.94	1.67
DVU2442	NA	Heat shock protein, Hsp20 family	3.64	**6.72**	0.72	1.40
DVU2986	*pspC*	Phage shock protein C	2.47	**4.48**	0.96	1.87
DVU2987	NA	Hypothetical protein	2.90	**5.42**	1.15	**2.22**
DVU2988	*pspA*	Phage shock protein A	3.51	**6.67**	1.33	**2.21**
DVU3093	*rdl*	Rubredoxin-like protein	1.14	**2.05**	0.48	0.90
DVU3094	*rbr*	Rubrerythrin	0.68	1.09	0.37	0.51
DVU3095	*perR*	Peroxide-responsive regulator PerR	2.07	**2.87**	0.69	1.25
DVU0198	NA	Minor capsid protein C, degenerate	-2.30	**-3.07**	-1.36	**-2.33**
DVU0199	NA	Conserved hypothetical protein	-3.13	**-4.22**	-1.66	**-2.98**
DVU0200	NA	Major head protein	-1.78	**-1.74**	-1.27	**-2.30**
DVU0201	NA	Hypothetical protein	-2.85	**-3.58**	-1.02	-1.85
DVU0202	NA	Holin	-2.71	**-3.29**	-1.07	-1.96
DVU0203	NA	Conserved hypothetical protein	-2.64	**-2.40**	-1.20	**-2.27**
DVU0204	NA	Lipoprotein, putative	-2.15	**-2.43**	-1.38	**-2.45**
DVU2568	*cpsA*	Peptidase, M20/M25/M40 family	-2.03	**-2.67**	-0.20	-0.31
DVU2569	*slyD*	Peptidyl-prolyl cis-trans isomerase	-1.41	**-2.05**	-1.15	**-2.21**

a: log_2 (treatment/control); b: Z score; c: no gene name has been assigned to this locus yet.

Table 3 Accumulation of amino acids in both control and NaCl treated *D. vulgaris* cells.

Amino acid	Control (nMol/mg, ±sd, n =5)	NaCl (nMol/mg, ±sd, n =5)	NaCl/control
Glutamate	10.25 ± 0.63	**82.82 ± 8.01**	**8.08**
Histidine	0.07 ± 0.00	0.20 ± 0.04	2.74
Serine	1.30 ± 0.12	2.54 ± 0.26	1.95
Betaine	1.40 ± 0.08	2.68 ± 0.58	1.92
Lysine	0.15 ± 0.01	0.28 ± 0.06	1.85
Glutamine	3.63 ± 0.38	**6.48 ± 0.51**	1.79
Alanine	9.89 ± 0.92	**17.53 ± 3.89**	**1.77**
Leucine	0.50 ± 0.01	0.79 ± 0.05	1.57
Threonine	1.45 ± 0.08	2.21 ± 0.17	1.52
Proline	1.46 ± 0.18	2.03 ± 0.35	1.39
Aspartate	7.41 ± 0.48	**10.21 ± 1.13**	1.38
Glycine	8.86 ± 1.70	**10.32 ± 3.16**	1.16
Isoleucine	0.69 ± 0.04	0.72 ± 0.02	1.05
Methionine	0.05 ± 0.00	0.05 ± 0.01	1.02
Arginine	0.94 ± 0.03	0.89 ± 0.03	0.94
Valine	5.39 ± 1.43	4.30 ± 1.14	0.80
Tyrosine	0.21 ± 0.00	0.13 ± 0.00	0.62
Phenylalanine	0.01 ± 0.00	0.01 ± 0.00	0.57
Tryptophan	0.005 ± 0.00	0.003 ± 0.00	0.55
Asparagine	ND	1.87 ± 0.41	

Where n and sd are abbreviations for the number of analytical measurements and the standard deviation, respectively.

Figure legends:

Fig. 1 Growth curves of *D. vulgaris* grown in the LS4D medium. The stock culture was activated overnight before inoculation. A. 1.0% of overnight activated culture was inoculated into the LS4D medium containing 0, 50, 100, 250, or 500 mM NaCl. B. 1.0% of overnight activated culture was inoculated into the LS4D medium containing 250 mM NaCl with or without 0.5% (wt/vol) yeast extract (YE) added, and the controls without NaCl or without YE were also included.

Fig. 2 Expression changes of representative genes of *D. vulgaris* involved in energy metabolism under salt adaptation in comparison with salt shock (120 min). Gene expression intensity data with the ratio of the NaCl treatment to the control converted to $\log_2$ values and visualized by JColorGrid software (Joachimiak et al., 2006). SA: salt adaptation; SS: salt shock at 120 min. No data are presented in the cells with gray color.

Fig. 3 Expression changes of representative genes of *D. vulgaris* involved in regulatory processes under salt adaptation in comparison with salt shock (120 min). Details for data presentation are the same as described in Fig. 2.

Fig. 4 Relief of salt inhibition of *D. vulgaris* by an external addition of amino acids, Glu, Ala, Leu, Trp and Lys in the LS4D medium under the control (A) and 250 mM NaCl stress (B) conditions. *D. vulgaris* cells were grown at 37^{o}C and their growth was monitored at OD_{600}.

Fig. 5 A general comparison of global transcriptromic profiling of *D. vulgaris* under salt adaptation and salt shock. Z score was set to |2.0|. Totally, 3321 ORFs were detected for both stress conditions, and all their Z scores were shown. The x-axis presents data from salt adaptation, and the y-axis from salt shock. I. Up-regulated genes under salt adaptation; II. Down-regulated genes under salt adaptation; III. Up-regulated genes under salt shock; IV. Down-regulated genes under salt shock; V. Up-regulated genes under both conditions; VI. Down-regulated genes under both conditions; VII. Insignificantly changed genes are located in the central rectangle. The number in each pair of brackets is the gene number in each above defined category.

Fig. 6 A comparison of expression of representative genes of *D. vulgaris* involved in efflux systems, the ATP synthase, sulfate reduction, and cell motility under salt adaptation and salt shock (120 min). Details for data presentation are the same as described in Fig. 2.

Fig. 7 A conceptual model of *D. vulgaris* Hildenborough responses to a long-term NaCl exposure. Color codes: red, up-regulation or increase; blue, down-regulation or decrease; gray, no significant change. A. Energy metabolism; B. General stress response; C. Protein, amino acid, and solute metabolism and transport.

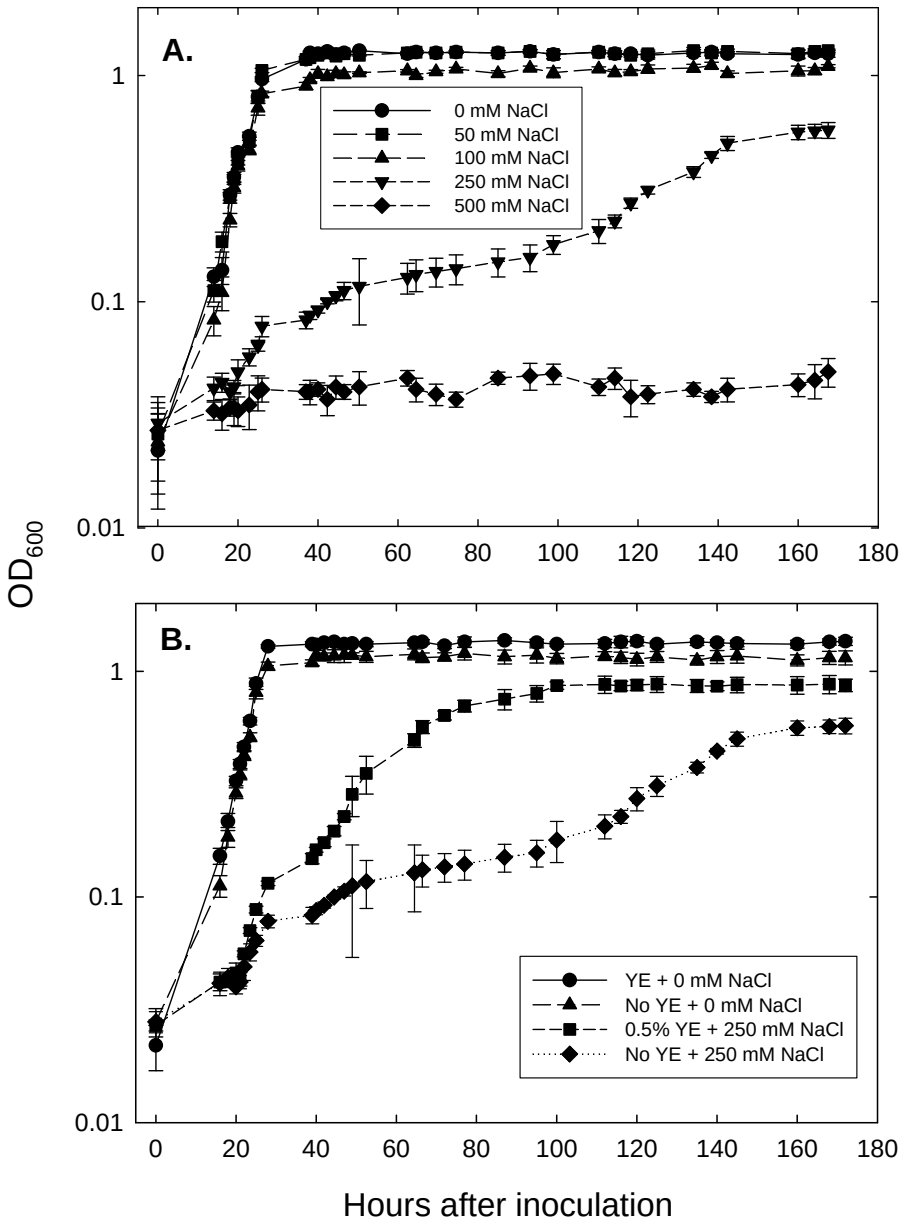

A.
0 mM NaCl
50 mM NaCl
100 mM NaCl
250 mM NaCl
500 mM NaCl
B.
YE + 0 mM NaCl
No YE + 0 mM NaCl
0.5% YE + 250 mM NaCl
No YE + 250 mM NaCl
OD600
Hours after inoculation
1
0.1
0.01
0
20
40
60
80
100
120
140
160
180

Fig. 1

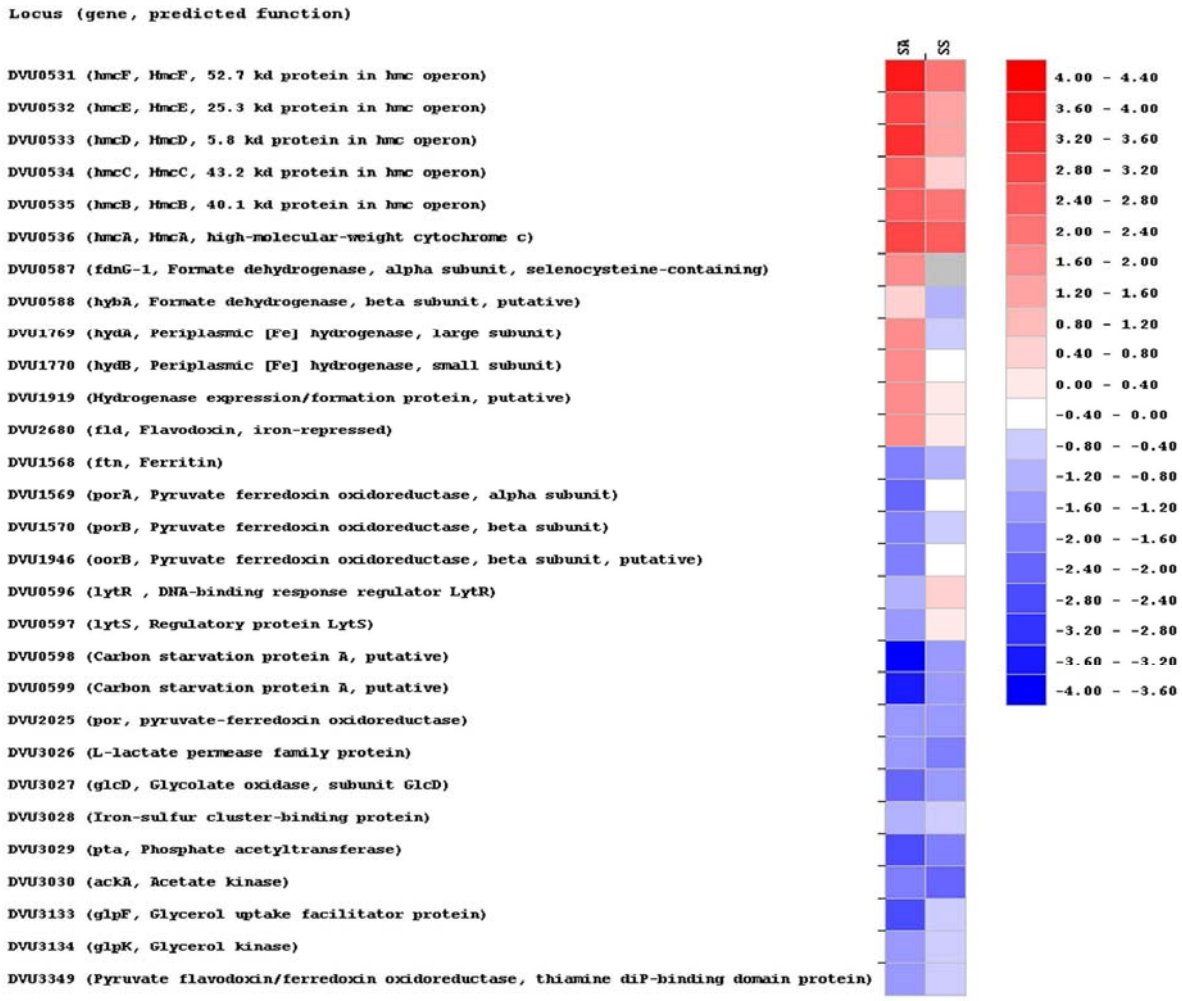
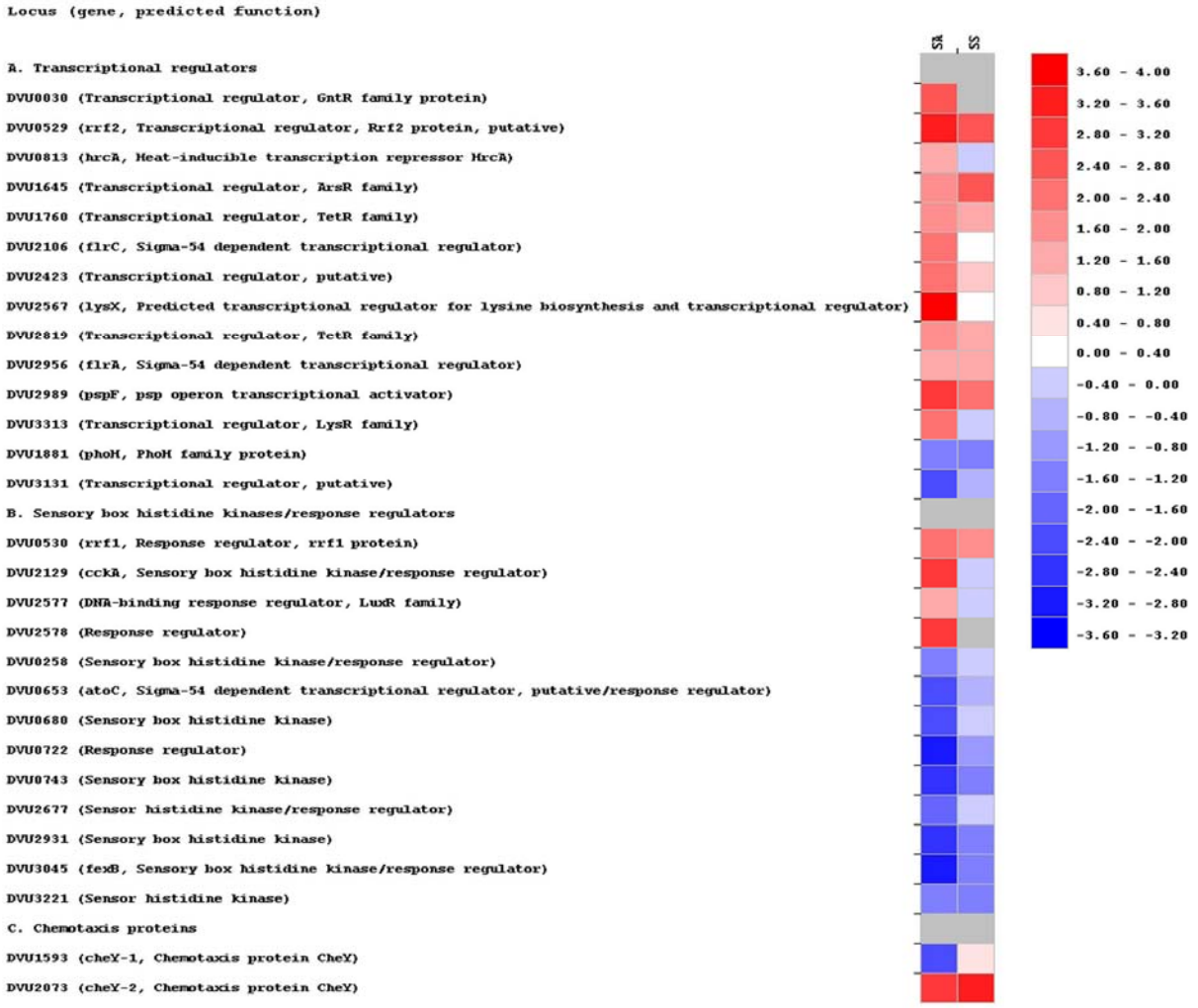

Locus (gene, predicted function)
SR
SS
DVU0531 (hmcF, HmcF, 52.7 kd protein in hmc operon)
DVU0532 (hmcE, HmcE, 25.3 kd protein in hmc operon)
DVU0533 (hmcD, HmcD, 5.8 kd protein in hmc operon)
DVU0534 (hmcC, HmcC, 43.2 kd protein in hmc operon)
DVU0535 (hmcB, HmcB, 40.1 kd protein in hmc operon)
DVU0536 (hmcA, HmcA, high-molecular-weight cytochrome c)
DVU0587 (fdnG-1, Formate dehydrogenase, alpha subunit, selenocysteine-containing)
DVU0588 (hybA, Formate dehydrogenase, beta subunit, putative)
DVU1769 (hydA, Periplasmic [Fe] hydrogenase, large subunit)
DVU1770 (hydB, Periplasmic [Fe] hydrogenase, small subunit)
DVU1919 (Hydrogenase expression/formation protein, putative)
DVU2680 (fld, Flavodoxin, iron-repressed)
DVU1568 (ftn, Ferritin)
DVU1569 (porA, Pyruvate ferredoxin oxidoreductase, alpha subunit)
DVU1570 (porB, Pyruvate ferredoxin oxidoreductase, beta subunit)
DVU1946 (oorB, Pyruvate ferredoxin oxidoreductase, beta subunit, putative)
DVU0596 (lytR , DNA-binding response regulator LytR)
DVU0597 (lytS, Regulatory protein LytS)
DVU0598 (Carbon starvation protein A, putative)
DVU0599 (Carbon starvation protein A, putative)
DVU2025 (por, pyruvate-ferredoxin oxidoreductase)
DVU3026 (L-lactate permease family protein)
DVU3027 (glcD, Glycolate oxidase, subunit GlcD)
DVU3028 (Iron-sulfur cluster-binding protein)
DVU3029 (pta, Phosphate acetyltransferase)
DVU3030 (ackA, Acetate kinase)
DVU3133 (glpF, Glycerol uptake facilitator protein)
DVU3134 (glpK, Glycerol kinase)
DVU3349 (Pyruvate flavodoxin/ferredoxin oxidoreductase, thiamine diP-binding domain protein)
4.00 - 4.40
3.60 - 4.00
3.20 - 3.60
2.80 - 3.20
2.40 - 2.80
2.00 - 2.40
1.60 - 2.00
1.20 - 1.60
0.80 - 1.20
0.40 - 0.80
0.00 - 0.40
-0.40 - 0.00
-0.80 - -0.40
-1.20 - -0.80
-1.60 - -1.20
-2.00 - -1.60
-2.40 - -2.00
-2.80 - -2.40
-3.20 - -2.80
-3.60 - -3.20
-4.00 - -3.60

Fig. 2


Locus (gene, predicted function)
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SS
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DVU0529 (rrf2, Transcriptional regulator, Rrf2 protein, putative)
DVU0813 (hrcA, Heat-inducible transcription repressor HrcA)
DVU1645 (Transcriptional regulator, ArsR family)
DVU1760 (Transcriptional regulator, TetR family)
DVU2106 (flrC, Sigma-54 dependent transcriptional regulator)
DVU2423 (Transcriptional regulator, putative)
DVU2567 (lysX, Predicted transcriptional regulator for lysine biosynthesis and transcriptional regulator)
DVU2819 (Transcriptional regulator, TetR family)
DVU2956 (flrA, Sigma-54 dependent transcriptional regulator)
DVU2989 (pspF, psp operon transcriptional activator)
DVU3313 (Transcriptional regulator, LysR family)
DVU1881 (phoH, PhoH family protein)
DVU3131 (Transcriptional regulator, putative)
B. Sensory box histidine kinases/response regulators
DVU0530 (rrf1, Response regulator, rrf1 protein)
DVU2129 (cckA, Sensory box histidine kinase/response regulator)
DVU2577 (DNA-binding response regulator, LuxR family)
DVU2578 (Response regulator)
DVU0258 (Sensory box histidine kinase/response regulator)
DVU0653 (atoC, Sigma-54 dependent transcriptional regulator, putative/response regulator)
DVU0680 (Sensory box histidine kinase)
DVU0722 (Response regulator)
DVU0743 (Sensory box histidine kinase)
DVU2677 (Sensor histidine kinase/response regulator)
DVU2931 (Sensory box histidine kinase)
DVU3045 (fexB, Sensory box histidine kinase/response regulator)
DVU3221 (Sensor histidine kinase)
C. Chemotaxis proteins
DVU1593 (cheY-1, Chemotaxis protein CheY)
DVU2073 (cheY-2, Chemotaxis protein CheY)
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3.20 - 3.60
2.80 - 3.20
2.40 - 2.80
2.00 - 2.40
1.60 - 2.00
1.20 - 1.60
0.80 - 1.20
0.40 - 0.80
0.00 - 0.40
-0.40 - 0.00
-0.80 - -0.40
-1.20 - -0.80
-1.60 - -1.20
-2.00 - -1.60
-2.40 - -2.00
-2.80 - -2.40
-3.20 - -2.80
-3.60 - -3.20

Fig. 3

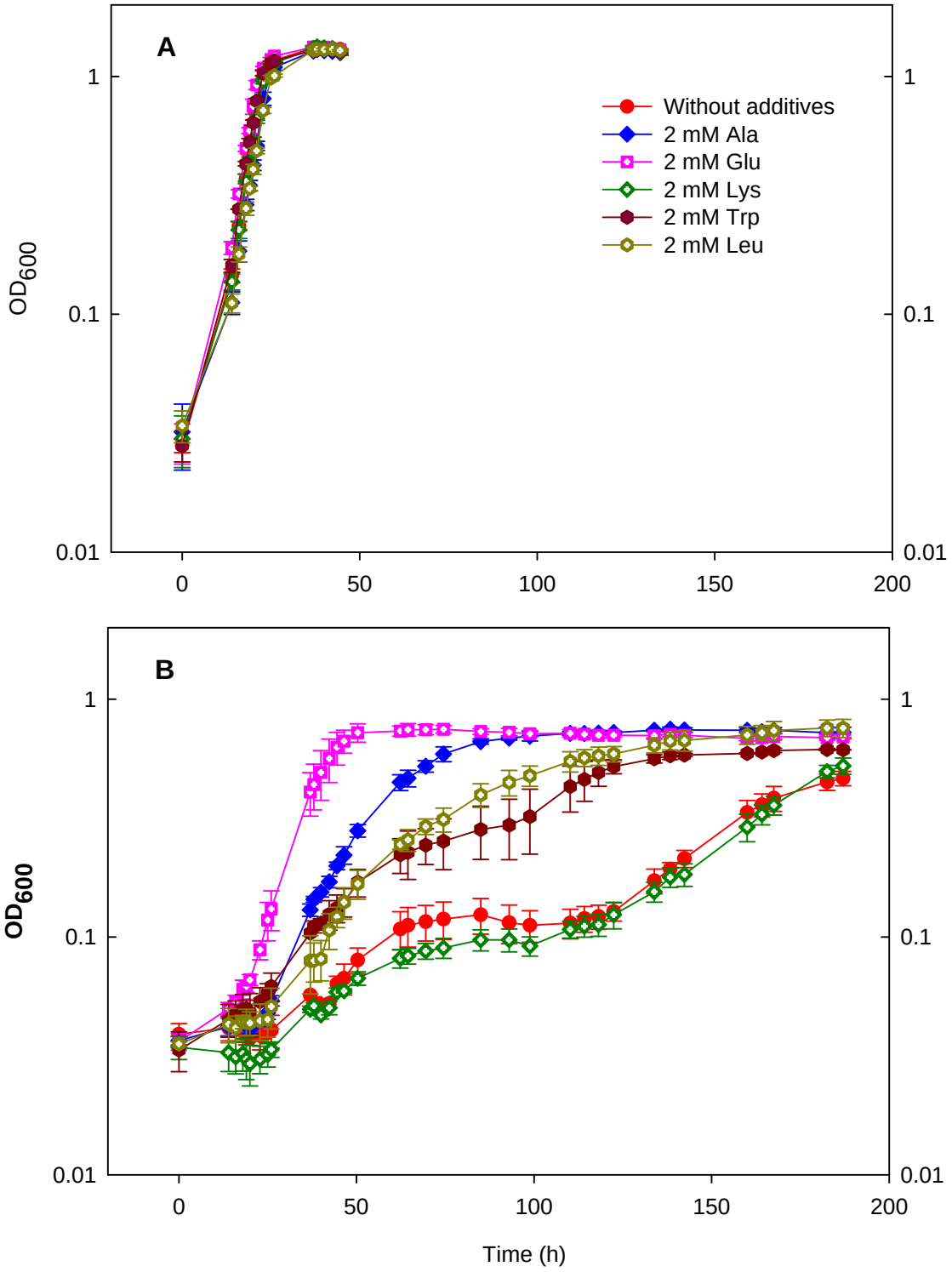

A
B
Without additives
2 mM Ala
2 mM Glu
2 mM Lys
2 mM Trp
2 mM Leu
OD600
Time (h)
1
0.1
0.01
0
50
100
150
200

Fig. 4

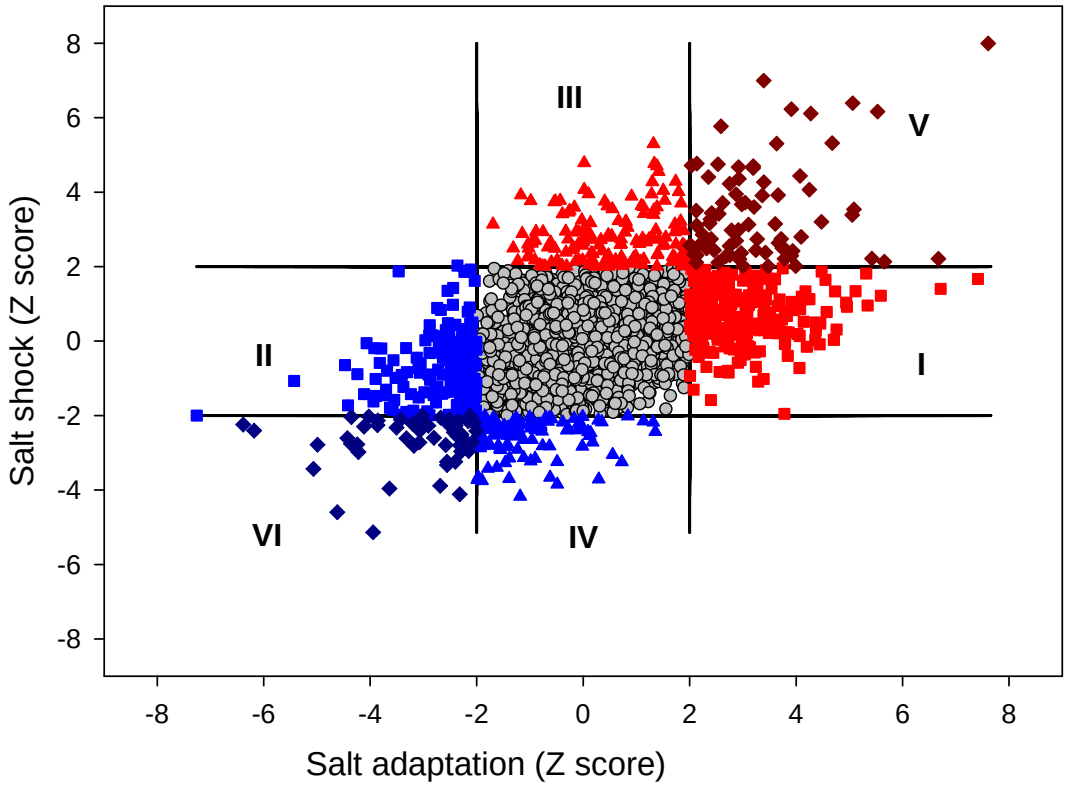
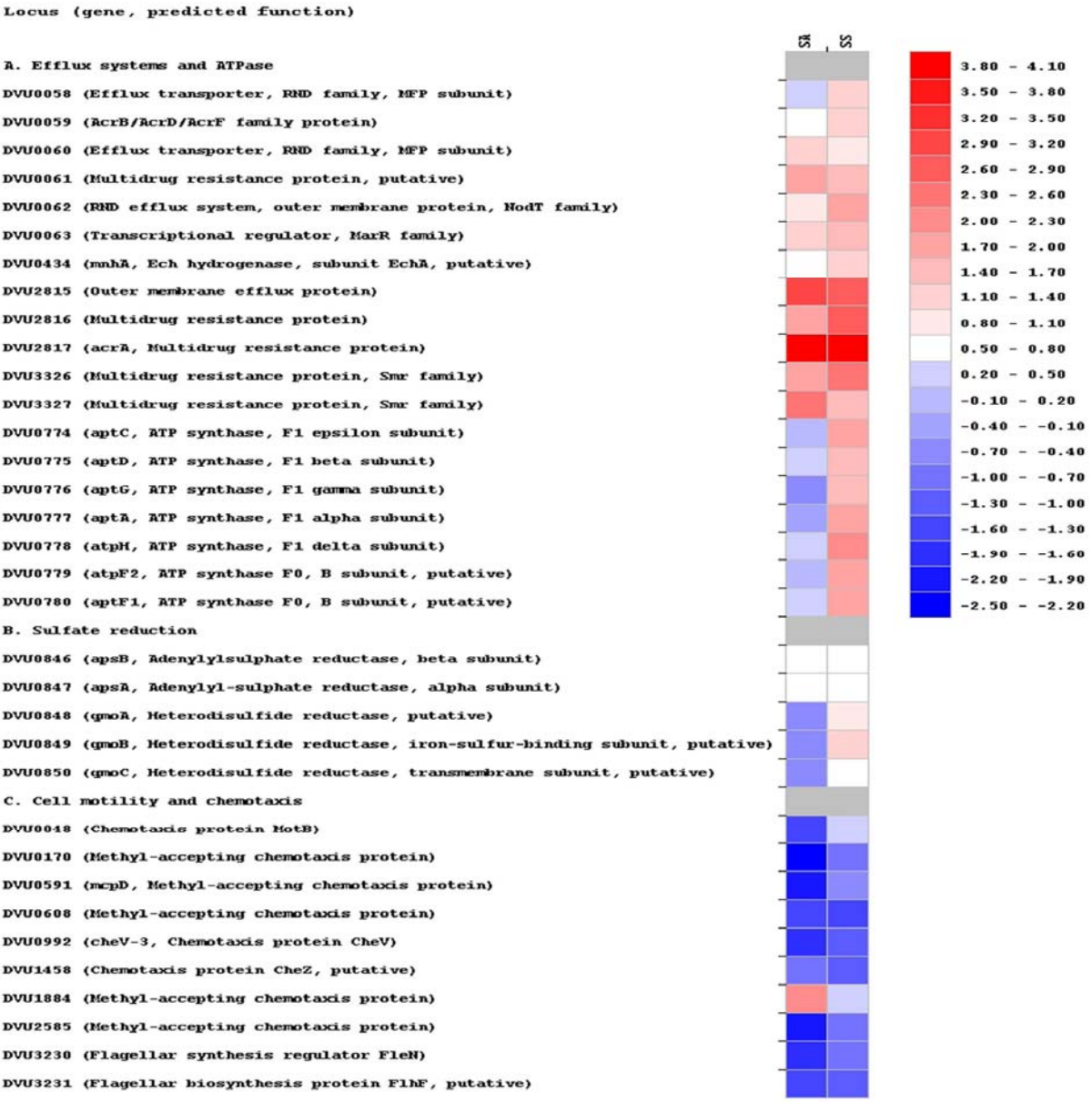

Salt shock (Z score)
Salt adaptation (Z score)
8
6
4
2
0
-2
-4
-6
-8
-8
-6
-4
-2
0
2
4
6
8
III
V
II
I
VI
IV

Fig. 5


Locus (gene, predicted function)
SR
SS
A. Efflux systems and ATPase
DVU0058 (Efflux transporter, RND family, MFP subunit)
DVU0059 (AcrB/AcrD/AcrF family protein)
DVU0060 (Efflux transporter, RND family, MFP subunit)
DVU0061 (Multidrug resistance protein, putative)
DVU0062 (RND efflux system, outer membrane protein, NodT family)
DVU0063 (Transcriptional regulator, MarR family)
DVU0434 (mnhA, Ech hydrogenase, subunit EchA, putative)
DVU2815 (Outer membrane efflux protein)
DVU2816 (Multidrug resistance protein)
DVU2817 (acrA, Multidrug resistance protein)
DVU3326 (Multidrug resistance protein, Smr family)
DVU3327 (Multidrug resistance protein, Smr family)
DVU0774 (aptC, ATP synthase, F1 epsilon subunit)
DVU0775 (aptD, ATP synthase, F1 beta subunit)
DVU0776 (aptG, ATP synthase, F1 gamma subunit)
DVU0777 (aptA, ATP synthase, F1 alpha subunit)
DVU0778 (atpH, ATP synthase, F1 delta subunit)
DVU0779 (atpF2, ATP synthase F0, B subunit, putative)
DVU0780 (aptF1, ATP synthase F0, B subunit, putative)
B. Sulfate reduction
DVU0846 (apsB, Adenylylsulphate reductase, beta subunit)
DVU0847 (apsA, Adenylyl-sulphate reductase, alpha subunit)
DVU0848 (qmoA, Heterodisulfide reductase, putative)
DVU0849 (qmoB, Heterodisulfide reductase, iron-sulfur-binding subunit, putative)
DVU0850 (qmoC, Heterodisulfide reductase, transmembrane subunit, putative)
C. Cell motility and chemotaxis
DVU0048 (Chemotaxis protein MotB)
DVU0170 (Methyl-accepting chemotaxis protein)
DVU0591 (mcpD, Methyl-accepting chemotaxis protein)
DVU0608 (Methyl-accepting chemotaxis protein)
DVU0992 (cheV-3, Chemotaxis protein CheV)
DVU1458 (Chemotaxis protein CheZ, putative)
DVU1884 (Methyl-accepting chemotaxis protein)
DVU2585 (Methyl-accepting chemotaxis protein)
DVU3230 (Flagellar synthesis regulator FleN)
DVU3231 (Flagellar biosynthesis protein FlhF, putative)
3.80 - 4.10
3.50 - 3.80
3.20 - 3.50
2.90 - 3.20
2.60 - 2.90
2.30 - 2.60
2.00 - 2.30
1.70 - 2.00
1.40 - 1.70
1.10 - 1.40
0.80 - 1.10
0.50 - 0.80
0.20 - 0.50
-0.10 - 0.20
-0.40 - -0.10
-0.70 - -0.40
-1.00 - -0.70
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-2.50 - -2.20

Fig. 6

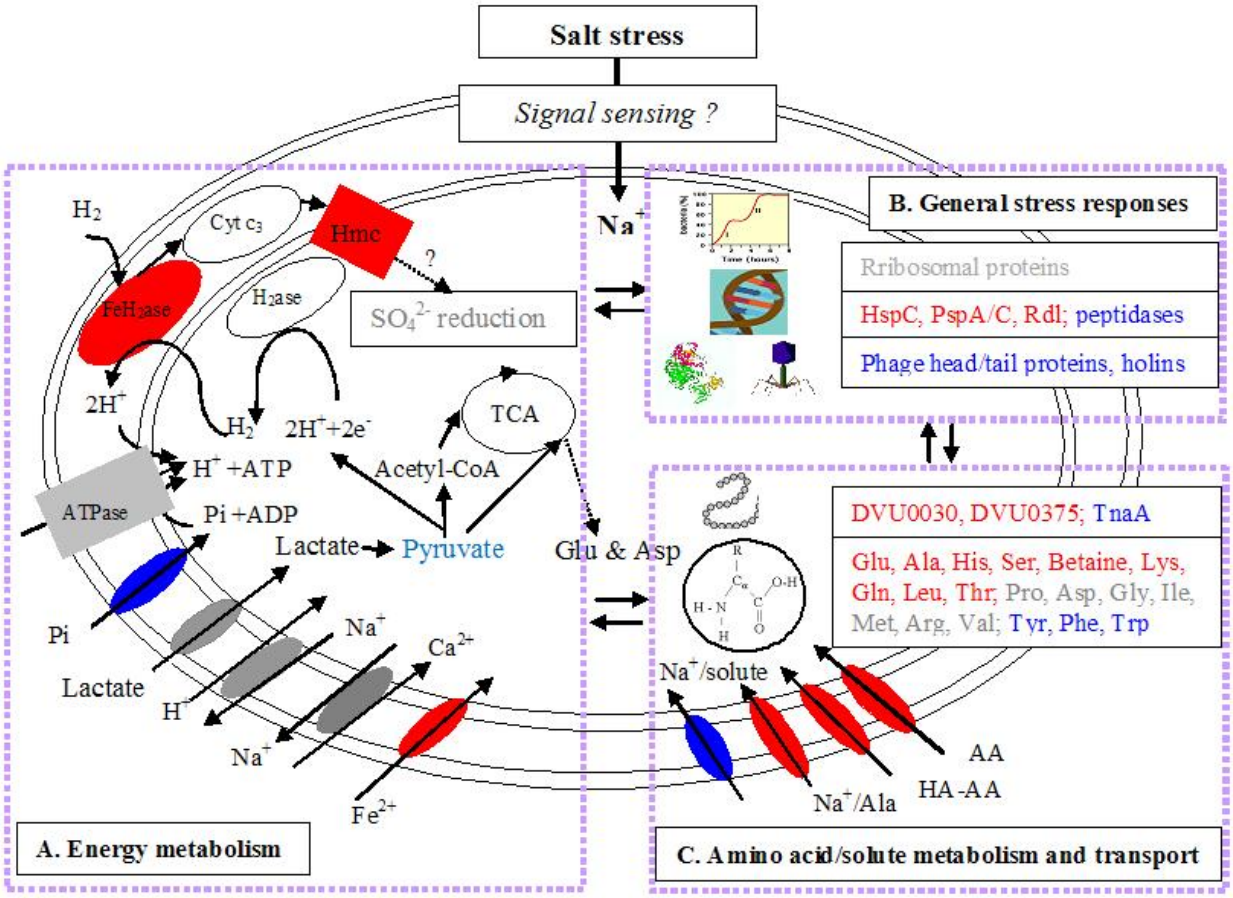
Salt stress
Signal sensing ?
Na+
B. General stress responses
Rribosomal proteins
HspC, PspA/C, Rdl; peptidases
Phage head/tail proteins, holins
H2
Cyt c3
Hmc
?
FeH2ase
H2ase
SO4 2- reduction
2H+
H2
2H++2e-
TCA
H+ +ATP
Acetyl-CoA
ATPase
Pi +ADP
Lactate
Pyruvate
Glu & Asp
DVU0030, DVU0375; TnaA
Glu, Ala, His, Ser, Betaine, Lys,
Gln, Leu, Thr; Pro, Asp, Gly, Ile,
Met, Arg, Val; Tyr, Phe, Trp
Pi
Lactate
H+
Na+
Ca2+
Na+
Fe2+
Na+/solute
AA
HA-AA
Na+/Ala
A. Energy metabolism
C. Amino acid/solute metabolism and transport

Fig. 7

Supplemental Data Summary

Fig. S1 Changes in gene expression profiling of *D. vulgaris* by functional category in response to salt adaptation.

Fig. S2 Correlation of microarray data and RT-PCR data for 12 randomly selected genes of *D. vulgaris*.

Table S1 Table 1 ORFs, used primers, and their product sizes for reverse transcription real time PCR assay.

Table S2 Table S2 Up-regulated ORFs under salt adaptation but no significant changes under salt shock.

Table S3 Table S3 Down-regulated ORFs under salt adaptation but no significant changes under salt shock.

Table S4 Table S4 Up-regulated ORFs under salt shock but no significant changes under salt adaptation.

Table S5 Table S5 Down-regulated ORFs under salt shock but no significant changes under salt adaptation.

Table S6 Table S6 Up-regulated ORFs under both salt shock and salt adaptation conditions.

Table S7 Table S7 Down-regulated ORFs under both salt shock and salt adaptation conditions.

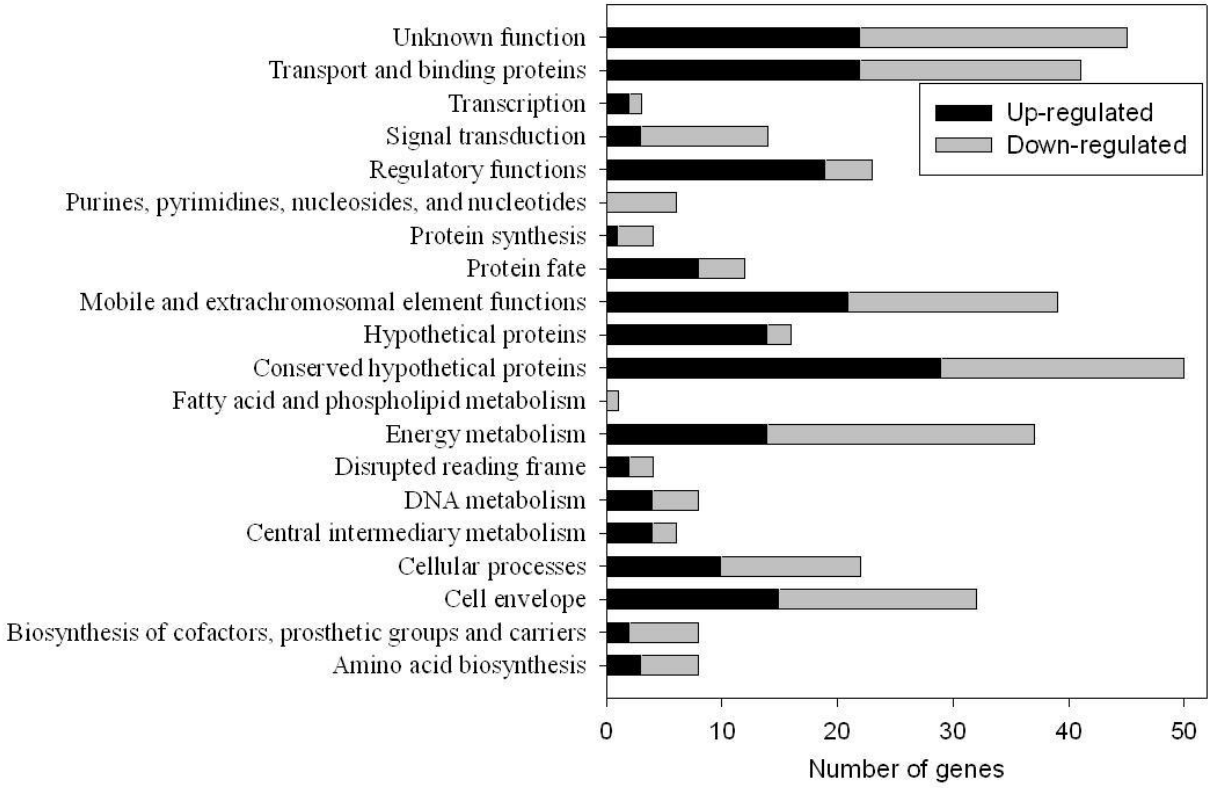

Fig. S1 Changes in gene expression profiling of *D. vulgaris* by functional category in response to salt adaptation. Inoculation of overnight activated *D. vulgaris* cells into the LS4D medium for the control, and LS4D + 250 mM NaCl for the treatment. Samples were taken at the mid-log phase (OD600 = ~0.25), and RNA and DNA were extracted, labeled, and co-hybridized with the *D. vulgaris* whole-genome microarray. Totally, 2647 ORFs were assigned to 20 functional categories based on the TIGR roles with 195 up-regulated and 184 down-regulated.

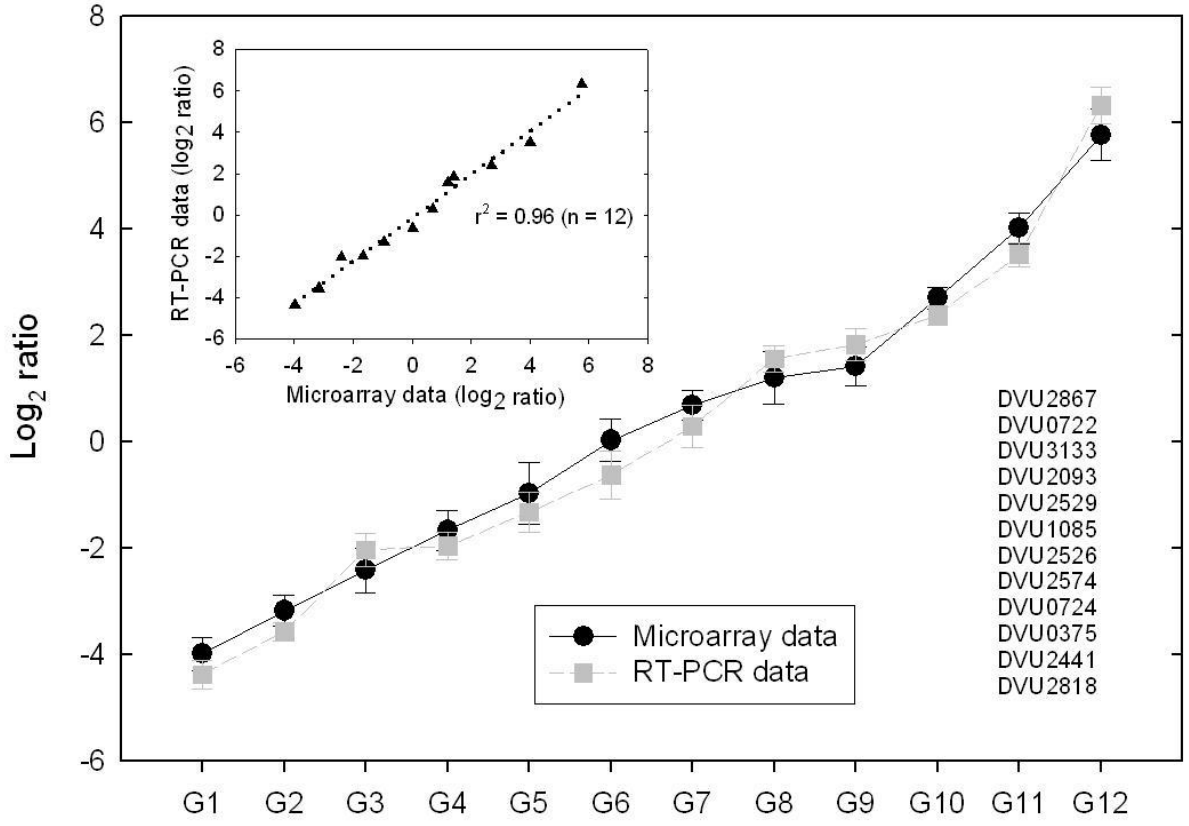

Fig. S2 Correlation of microarray data and RT-PCR data for 12 randomly selected genes of *D. vulgaris*. 12 genes (G1-12) with a range of expression levels were randomly selected for RT-PCR to quantitatively verify the microarray data using specific primers (Table S1). The same RNA prepared for microarray hybridization was used.

Summplemental Data - Table S1				
Table 1 ORFs, used primers, and their product sizes for reverse transcription real time PCR assay				
Locus tag	Forward primer	Reverse primer	Predicted function	Size (bp)
DVU2867	GACTACGTGCTGGGCTTTGT	GCGGCGAAGTAGAAGAGGAA	Holin	98
DVU0722	TCGAGCGTATCATCCATTCA	CGCTGAGTGACGAAGTGAGT	Response regulator	99
DVU3133	GCCATCGTCTTTCTCGACTT	ATGTGGTTATGGCAGGGAAG	Glycerol uptake facilitator protein	100
DVU2093	CTCCATTTGCAAGACCATCC	ATCTCCATGCGCTTGTCTTC	ThiH protein	99
DVU2529	TCTCGACAACCTGTCACACC	TAGAGGGACTTGCCCACTT	Phosphoglycerate kinase	102
DVU1085	ACGCAAGGCCATAGACAGTT	CGCGTCTCACGCTCTATCTC	Phosphate transport system protein PhoU	99
DVU2526	ACTTCTTCGACCCTGAGACG	TATCTGTTCCGGGTTCACCT	Periplasmic [NiFe] hydrogenase, large subunit	101
DVU2574	TCGGAGATAGAGGTCTGTTCG	GCACAGCACAGTGCAAGC	Ferrous ion transport protein, putative	99
DVU0724	ATGACCACGCTTCAGCTTTT	GGCGGATGGTAAGGTAGATG	Sodium/alanine symporter family protein	100
DVU0375	GAAGAACGACGTGCTCAACC	ACGTCCTTGATCTCGCAGAC	Glu/Leu/Phe/Val dehydrogenase family protein	102
DVU2441	GCGTAACCCCTGAAGACATC	GCTCCTGCCTGAACATCCT	Heat shock protein, Hsp20 family	99
DVU2818	CAAGGATGTTGTGCTGCCTA	CACGGGAAAGTGTGGTGAAC	Hypothetical protein	99

Supplemental Table S2-7

Table S2 Up-regulated ORFs under salt adaptation but no significant changes under salt shock

	Salt shock			Salt adaptation		
Locus tag	Ratio	Zscore		Ratio	Zscore	Annotation
DVU0016	0.39	0.78		2.11	2.75	hypothetical protein
DVU0024	0.93	1.68		2.38	3.45	conserved hypothetical protein
DVU0086	0.21	0.40		1.06	2.00	hypothetical protein
DVU0103	0.98	1.69		1.94	3.19	cation ABC transporter, ATP-binding protein, putative
DVU0115	0.74	1.19		1.58	2.01	shikimate 5-dehydrogenase
DVU0122	-1.30	-1.96		2.07	3.78	hypothetical protein
DVU0124	0.73	1.06		1.38	2.33	hypothetical protein
DVU0163	0.24	0.44		1.35	2.48	lipoprotein, putative
DVU0186	-0.32	-0.51		1.65	2.96	conserved hypothetical protein
DVU0196	0.66	1.25		1.53	2.35	hypothetical protein
DVU0225	0.02	0.04		1.59	3.05	hypothetical protein
DVU0236	1.00	1.76		1.92	3.24	site-specific recombinase, phage integrase family
DVU0297	-0.17	-0.31		1.32	2.44	hypothetical protein
DVU0303	1.01	1.65		3.28	4.55	hypothetical protein
DVU0361	0.12	0.20		1.69	2.35	acetolactate synthase III, small subunit, putative
DVU0365	0.70	1.34		2.92	4.18	conserved hypothetical protein
DVU0375	0.70	1.12		2.70	3.69	Glu/Leu/Phe/Val dehydrogenase family protein
DVU0411	0.52	0.96		1.76	3.05	heptosyltransferase family protein
DVU0419	-0.14	-0.24		1.63	3.01	carboxynorspermidine decarboxylase
DVU0420	0.19	0.33		1.69	2.58	hypothetical protein
DVU0444	0.79	1.37		2.18	2.48	CBS domain protein
DVU0473	0.18	0.33		1.45	2.63	hypothetical protein
DVU0497	0.02	0.03		2.98	4.71	hypothetical protein
DVU0532	1.13	1.83		2.76	2.25	hmc operon protein 5
DVU0534	0.38	0.67		2.42	3.83	hmc operon protein 3
DVU0559	-0.11	-0.20		1.90	2.75	lipoprotein, putative
DVU0572	0.46	0.89		1.90	3.41	hypothetical protein
DVU0586	-0.56	-1.09		2.13	3.29	hypothetical protein
DVU0593	0.87	1.68		2.74	3.54	L-lysine exporter, putative
DVU0620	-0.22	-0.43		1.37	2.17	endoribonuclease, L-PSP family
DVU0724	0.53	1.04		1.42	2.69	sodium/alanine symporter family protein
DVU0753	0.27	0.51		1.17	2.09	amino acid ABC transporter, ATP-binding protein
DVU0758	0.11	0.21		1.66	3.08	hypothetical protein
DVU0772	-0.85	-1.58		2.25	2.40	hypothetical protein
DVU0781	0.43	0.84		1.56	2.57	hypothetical protein
DVU0782	0.51	0.85		1.15	2.04	hypothetical protein
DVU0813	-0.06	-0.12		1.35	2.16	heat-inducible transcription repressor HrcA
DVU0948	0.85	1.46		1.75	2.51	conserved hypothetical protein
DVU0949	-0.12	-0.14		3.01	3.10	conserved domain protein
DVU0999	0.46	0.89		1.46	2.08	thio:disulfide interchange protein, putative
DVU1068	0.42	0.78		2.70	4.57	branched-chain amino acid ABC transporter, permease protein
DVU1072	0.65	1.25		1.62	2.61	conserved hypothetical protein
DVU1091	-0.01	-0.01		1.26	2.15	conserved hypothetical protein
DVU1105	0.22	0.33		1.34	2.03	hypothetical protein
DVU1106	0.45	0.81		2.18	2.70	hypothetical protein
DVU1114	0.18	0.24		1.97	2.47	virion morphogenesis protein
DVU1115	0.46	0.83		2.95	3.96	conserved hypothetical protein
DVU1118	-0.36	-0.58		2.51	2.91	conserved hypothetical protein
DVU1121	0.67	1.23		2.26	3.33	hypothetical protein
DVU1122	0.39	0.57		3.12	3.60	portal protein, putative

DVU1123	0.32	0.58		3.03	3.73	conserved domain protein
DVU1124	0.92	1.34		3.47	5.12	hypothetical protein
DVU1130	0.43	0.82		1.72	2.48	DNA-binding protein
DVU1132	0.62	1.13		1.90	3.15	conserved hypothetical protein
DVU1140	0.51	0.97		1.95	3.12	bacteriophage transposase A protein, putative
DVU1143	0.20	0.33		2.57	3.01	hypothetical protein
DVU1154	0.94	1.81		4.12	5.32	hypothetical protein
DVU1166	-0.36	-0.69		2.05	3.24	hypothetical protein
DVU1173	0.04	0.08		1.64	2.60	integral membrane protein MviN
DVU1177	-0.09	-0.14		3.28	2.99	hypothetical protein
DVU1178	-0.44	-0.83		2.00	2.56	hypothetical protein
DVU1179	-0.05	-0.09		2.45	4.44	aldehyde:ferredoxin oxidoreductase, tungsten-containing
DVU1212	1.07	1.26		1.40	2.43	fxsA protein
DVU1256	0.18	0.35		1.49	2.62	heptosyltransferase family protein
DVU1410	0.20	0.40		1.11	2.03	conserved domain protein
DVU1473	0.81	1.22		1.15	2.15	hypothetical protein
DVU1475	0.28	0.54		1.79	2.47	PhoU family protein
DVU1479	0.33	0.53		1.21	2.29	conserved hypothetical protein
DVU1489	-0.12	-0.20		1.22	2.26	hypothetical protein
DVU1490	0.33	0.59		2.59	2.76	tail tape measure protein, putative
DVU1499	0.87	1.61		1.31	2.09	hypothetical protein
DVU1513	0.79	1.12		1.76	2.21	conserved hypothetical protein
DVU1514	0.76	1.31		1.81	2.65	hypothetical protein
DVU1515	0.24	0.35		1.54	2.03	type II DNA modification methyltransferase, putative
DVU1517	0.42	0.70		1.62	2.59	transcriptional regulator cII, putative
DVU1525	0.76	1.10		1.50	2.67	conserved domain protein
DVU1637	0.91	1.47		1.78	3.20	hypothetical protein
DVU1638	0.51	0.92		1.62	2.76	conserved domain protein
DVU1639	0.98	1.91		1.29	2.29	conserved domain protein
DVU1654	-0.15	-0.27		2.46	3.32	site-specific recombinase, phage integrase family
DVU1661	0.53	1.02		1.16	2.21	hypothetical protein
DVU1699	1.00	1.85		2.20	3.05	hypothetical protein
DVU1700	0.68	1.19		2.82	4.11	metallo-beta-lactamase family protein
DVU1712	0.53	0.47		2.92	4.46	hypothetical protein
DVU1713	0.35	0.33		1.45	2.47	hypothetical protein
DVU1716	0.73	1.12		1.93	2.42	hypothetical protein
DVU1717	0.20	0.34		1.58	2.72	hypothetical protein
DVU1719	1.42	1.83		1.83	2.66	conserved domain protein
DVU1720	0.49	0.70		1.85	3.38	hypothetical protein
DVU1723	0.24	0.34		2.34	3.21	hypothetical protein
DVU1740	0.48	0.81		2.54	3.81	hypothetical protein
DVU1741	0.01	0.02		2.52	4.36	hypothetical protein
DVU1750	0.24	0.47		1.53	2.75	hypothetical protein
DVU1757	0.12	0.17		2.76	3.00	site-specific recombinase, phage integrase family
DVU1763	0.88	1.67		2.69	2.73	hypothetical protein
DVU1767	0.67	1.07		1.78	2.90	radical SAM domain protein
DVU1769	-0.69	-1.30		1.57	2.07	periplasmic [Fe] hydrogenase, large subunit
DVU1770	-0.07	-0.12		1.62	2.19	periplasmic [Fe] hydrogenase, small subunit
DVU1884	0.39	0.57		1.91	2.35	methyl-accepting chemotaxis protein
DVU1905	0.39	0.72		1.56	2.40	hypothetical protein
DVU1919	0.08	0.13		1.66	2.98	hydrogenase expression/formation protein, putative
DVU1965	0.62	1.05		1.76	3.25	hypothetical protein
DVU1968	0.75	1.41		1.83	2.27	oxidoreductase, putative
DVU2003	0.58	1.13		2.69	4.17	transposase, IS5 family, truncation
DVU2007	0.11	0.21		2.04	3.67	nuclease, putative

DVU2017	-0.13	-0.22		1.56	2.83	ISDvu5, transposase
DVU2067	1.02	1.78		1.68	2.80	GGDEF domain protein
DVU2070	0.81	1.33		3.39	4.73	TPR domain protein
DVU2080	0.07	0.12		1.44	2.52	hypothetical protein
DVU2101	1.06	1.95		3.87	3.75	conserved hypothetical protein
DVU2106	0.32	0.50		2.09	3.61	sigma-54 dependent transcriptional regulator
DVU2107	0.46	0.87		2.32	2.20	hypothetical protein
DVU2122	-0.09	-0.14		2.44	2.66	type II/IV secretion system protein
DVU2125	-0.22	-0.31		2.04	3.22	TPR domain protein
DVU2127	0.14	0.23		2.43	2.94	von Willebrand factor type A domain protein
DVU2129	-0.21	-0.36		2.88	3.04	sensory box histidine kinase/response regulator
DVU2145	0.87	1.62		1.71	3.00	chloramphenicol acetyltransferase, putative
DVU2146	-0.39	-0.73		2.45	4.06	hypothetical protein
DVU2154	0.73	1.13		2.40	4.25	tail assembly protein, putative
DVU2155	0.08	0.14		1.64	2.07	hypothetical protein
DVU2156	0.69	0.78		2.52	3.79	hypothetical protein
DVU2158	0.73	1.23		3.11	3.12	hypothetical protein
DVU2159	0.55	0.92		2.85	4.95	hypothetical protein
DVU2160	0.58	0.99		2.15	2.79	hypothetical protein
DVU2161	0.41	0.55		2.77	3.48	hypothetical protein
DVU2163	-0.15	-0.23		1.79	2.10	hypothetical protein
DVU2164	0.18	0.32		2.07	3.19	lipoprotein, putative
DVU2165	-0.58	-1.02		2.37	3.39	lysozyme, putative
DVU2167	-0.09	-0.17		2.45	3.13	hypothetical protein
DVU2172	-0.19	-0.29		2.07	2.37	hypothetical protein
DVU2181	0.46	0.78		1.49	2.02	antirepressor, putative
DVU2182	0.99	1.41		4.18	3.54	hypothetical protein
DVU2183	0.82	1.13		5.05	3.43	hypothetical protein
DVU2189	0.16	0.28		1.36	2.18	transcriptional regulator cII, putative
DVU2204	0.27	0.47		1.81	3.22	tryptophanase
DVU2218	0.33	0.61		2.17	2.93	GTP-binding protein, putative
DVU2247	0.18	0.31		2.77	4.76	antioxidant, AhpC/Tsa family
DVU2249	0.46	0.76		2.75	3.51	hypothetical protein
DVU2266	0.00	0.00		3.04	3.79	hypothetical protein
DVU2278	0.70	1.03		2.82	4.94	membrane protein, putative
DVU2283	0.99	1.80		1.38	2.14	hypothetical protein
DVU2327	0.12	0.24		2.39	3.81	hypothetical protein
DVU2341	0.78	1.18		1.56	2.76	amino acid ABC transproter, permease protein, His/Glu/Gln/Arg/opine family
DVU2344	0.23	0.37		2.85	3.31	hypothetical protein
DVU2358	1.02	1.67		1.75	2.68	hypothetical protein
DVU2372	0.45	0.84		1.21	2.29	hypothetical protein
DVU2423	0.90	1.66		2.41	3.60	transcriptional regulator, putative
DVU2426	0.16	0.29		2.23	2.83	hypothetical protein
DVU2441	0.94	1.67		4.01	7.42	heat shock protein, Hsp20 family
DVU2442	0.72	1.40		3.64	6.72	heat shock protein, Hsp20 family
DVU2450	0.32	0.61		1.21	2.19	conserved hypothetical protein
DVU2480	-0.08	-0.15		2.75	4.20	hypothetical protein
DVU2494	0.17	0.31		1.70	3.07	peptidase, M48 family
DVU2542	0.28	0.45		1.88	3.30	hypothetical protein
DVU2556	-0.09	-0.17		1.45	2.25	hypothetical protein
DVU2559	0.35	0.52		2.47	4.14	adenosylmethionine--8-amino-7-oxononanoate aminotransferase
DVU2560	1.22	1.94		3.29	2.03	conserved domain protein
DVU2567	0.20	0.33		3.48	3.32	conserved hypothetical protein
DVU2573	1.18	1.90		1.94	3.43	hypothetical protein

DVU2577	0.02	0.04		1.48	2.53	DNA-binding response regulator, LuxR family
DVU2595	0.36	0.63		1.83	3.04	hypothetical protein
DVU2602	0.27	0.38		2.62	3.65	conserved domain protein
DVU2611	0.62	1.22		3.44	5.59	conserved hypothetical protein
DVU2637	0.10	0.19		2.95	4.02	HAMP domain protein
DVU2671	0.80	1.54		1.32	2.28	HDIG/HD/KH domain protein
DVU2680	0.18	0.29		1.90	3.31	flavodoxin
DVU2681	0.79	1.34		2.50	2.71	hypothetical protein
DVU2688	0.38	0.70		1.59	2.35	bacteriophage transposase A protein
DVU2690	0.59	1.08		1.92	2.42	hypothetical protein
DVU2692	0.31	0.53		1.71	2.09	conserved domain protein
DVU2695	0.33	0.50		2.83	4.38	conserved hypothetical protein
DVU2697	0.92	1.25		1.15	2.01	hypothetical protein
DVU2700	0.35	0.63		1.44	2.24	hypothetical protein
DVU2701	0.35	0.62		2.10	2.40	hypothetical protein
DVU2704	0.06	0.11		2.24	2.72	conserved hypothetical protein
DVU2706	0.24	0.31		2.21	2.75	conserved hypothetical protein
DVU2720	0.57	1.10		1.78	2.09	hypothetical protein
DVU2724	0.36	0.65		2.09	2.92	phage baseplate assembly protein V, putative
DVU2725	0.97	1.48		1.87	3.27	membrane protein, putative
DVU2726	-0.16	-0.31		1.30	2.36	hypothetical protein
DVU2727	0.24	0.46		2.40	3.14	conserved hypothetical protein
DVU2740	0.61	0.96		3.18	5.34	high-affinity branched-chain amino acid ABC transporter, ATP-binding protein
DVU2742	0.93	1.76		1.79	2.08	high-affinity branched chain amino acid ABC transporter, permease protein
DVU2755	0.60	1.12		1.49	2.66	conserved hypothetical protein
DVU2769	0.30	0.56		1.42	2.49	conserved hypothetical protein
DVU2775	1.07	1.94		1.32	2.10	hypothetical protein
DVU2786	0.17	0.23		2.48	3.86	hypothetical protein
DVU2789	0.13	0.22		2.21	3.49	Gpr1/Fun34/YaaH family protein
DVU2808	-0.04	-0.08		2.61	3.79	TonB domain protein
DVU2827	0.35	0.61		1.41	2.06	sigma-54 dependent transcriptional regulator
DVU2828	0.11	0.21		2.18	2.30	site-specific recombinase, phage integrase family
DVU2856	0.09	0.17		2.05	2.91	conserved domain protein
DVU2874	0.69	1.16		1.70	2.90	hypothetical protein
DVU2917	0.34	0.60		1.33	2.42	UDP-3-0-acyl N-acetylglucosamine deacetylase
DVU2919	0.11	0.20		1.38	2.50	hypothetical protein
DVU2986	0.96	1.87		2.47	4.48	phage shock protein C
DVU3021	0.90	1.75		1.77	2.53	HDIG domain protein
DVU3081	0.20	0.34		1.45	2.19	membrane protein, putative
DVU3083	0.24	0.47		1.31	2.16	hypothetical protein
DVU3093	0.48	0.90		1.14	2.05	rubredoxin-like protein
DVU3095	0.69	1.25		2.07	2.87	transcriptional regulator, Fur family
DVU3105	-0.37	-0.69		1.22	2.32	hypothetical protein
DVU3110	0.87	1.69		1.84	2.33	L-aspartate oxidase, putative
DVU3115	0.48	0.94		2.03	3.66	hypothetical protein
DVU3124	0.64	0.86		2.16	4.08	hypothetical protein
DVU3129	0.14	0.26		2.50	3.69	hypothetical protein
DVU3138	0.56	1.05		2.18	3.92	hypothetical protein
DVU3143	-0.48	-0.84		2.15	2.73	iron-sulfur cluster-binding protein
DVU3194	0.74	1.10		1.45	2.32	GTP-binding protein EngA
DVU3251	0.17	0.29		2.64	4.37	membrane protein, HPP family
DVU3285	0.88	1.38		2.17	2.40	hypothetical protein
DVU3297	0.35	0.65		1.34	2.48	tryptophan-specific transport protein

DVU3311	0.41	0.77		1.35	2.20	hypothetical protein
DVU3313	0.08	0.14		2.32	4.07	transcriptional regulator, LysR family
DVU3325	0.78	1.49		1.62	2.74	hypothetical protein
DVU3328	0.67	1.25		1.44	2.65	hypothetical protein
DVU3332	0.90	1.67		1.62	2.54	heavy metal translocating P-type ATPase
DVU3333	0.72	0.91		2.63	2.23	hypothetical protein
DVU3354	0.27	0.50		2.21	2.30	hypothetical protein
DVUA0003	1.56	1.53		1.40	2.16	hypothetical protein
DVUA0018	-0.23	-0.40		2.14	3.84	hypothetical protein
DVUA0026	-0.74	-0.80		2.15	2.72	hypothetical protein
DVUA0028	0.54	0.79		1.99	3.09	hypothetical protein
DVUA0085	0.32	0.38		2.08	3.08	conserved hypothetical protein
DVUA0103	0.16	0.29		1.38	2.44	type III secretion protein, HrpO family
DVUA0132	-0.80	-0.93		1.21	2.01	CRISPR-associated protein, TM1801 family
DVUA0140	0.44	0.49		4.14	3.56	hypothetical protein
DVUA0141	0.06	0.09		1.32	2.23	hypothetical protein

Table S3 Down-regulated ORFs under salt adaptation but no significant changes under salt shock

	Salt shock			Salt adaptation		
Locus tag	Ratio	Zscore		Ratio	Zscore	Annotation
DVU0818	1.20	2.03		-1.53	-2.36	conserved domain protein
DVU0027	-1.10	-1.95		-1.63	-2.22	membrane protein, putative
DVU0048	0.41	0.79		-1.33	-2.17	chemotaxis protein MotB
DVU0053	-0.25	-0.47		-1.26	-2.10	sulfate permease, putative
DVU0075	-0.47	-0.90		-2.26	-2.91	aminotransferase, DegT/DnrJ/EryC1/StrS family
DVU0077	-0.27	-0.54		-1.39	-2.26	conserved hypothetical protein
DVU0116	0.15	0.29		-1.65	-2.58	polysaccharide deacetylase family protein
DVU0127	-0.51	-0.99		-1.96	-3.52	membrane protein, putative
DVU0134	-0.06	-0.11		-1.61	-2.35	glycosyl transferase, group 2 family protein
DVU0150	0.19	0.32		-1.74	-2.27	membrane protein, putative
DVU0170	-1.00	-1.58		-2.44	-3.55	methyl-accepting chemotaxis protein
DVU0189	-0.27	-0.51		-2.72	-3.56	phage/plasmid primase, P4 family
DVU0192	0.22	0.42		-1.69	-2.88	adenine specific DNA methyltransferase, putative
DVU0194	-0.34	-0.59		-2.47	-3.82	terminase, large subunit, putative
DVU0195	-0.11	-0.21		-1.43	-2.37	hypothetical protein
DVU0201	-1.02	-1.85		-2.85	-3.58	hypothetical protein
DVU0202	-1.07	-1.96		-2.71	-3.29	holin
DVU0214	-0.63	-1.23		-2.06	-3.38	tail/DNA circulation protein, putative
DVU0216	-0.79	-1.43		-2.45	-3.24	phage baseplate assembly protein V, putative
DVU0217	-0.84	-1.62		-2.26	-3.94	tail protein, putative
DVU0218	-0.77	-1.46		-1.35	-2.32	tail protein, putative
DVU0221	-0.55	-0.96		-1.91	-2.87	tail fiber assembly protein, putative
DVU0233	0.51	0.97		-2.18	-2.45	hypothetical protein
DVU0235	1.00	1.92		-1.33	-2.11	hypothetical protein
DVU0254	-0.35	-0.63		-1.32	-2.01	hypothetical protein
DVU0258	-0.02	-0.03		-1.43	-2.23	sensory box histidine kinase/response regulator
DVU0277	-0.17	-0.33		-1.40	-2.29	transcriptional regulator, AraC family
DVU0293	-0.55	-1.02		-1.73	-2.87	prokaryotic dksA/traR C4-type zinc finger family protein
DVU0298	-0.25	-0.45		-1.49	-2.09	hypothetical protein
DVU0305	-1.01	-1.87		-1.30	-2.01	ferredoxin II
DVU0339	-0.40	-0.74		-1.70	-2.25	D-isomer specific 2-hydroxyacid dehydrogenase family protein
DVU0340	-0.72	-1.12		-1.26	-2.01	acetyltransferase, CysE/LacA/LpxA/NodL family
DVU0341	-0.98	-1.88		-2.29	-2.93	3-deoxy-D-manno-octulosonate cytidylyltransferase
DVU0348	-0.48	-0.84		-2.12	-3.31	hypothetical protein

DVU0350	-0.33	-0.63		-1.76	-2.25	spore coat polysaccharide biosynthesis protein spsF
DVU0372	0.33	0.50		-2.31	-2.08	membrane protein, putative
DVU0424	-0.04	-0.07		-1.24	-2.08	cardiolipin synthetase
DVU0425	-0.11	-0.20		-2.09	-3.79	hypothetical protein
DVU0446	-0.11	-0.19		-2.55	-3.33	sodium/solute symporter family protein
DVU0591	-0.53	-1.04		-2.02	-2.87	methyl-accepting chemotaxis protein
DVU0650	-1.11	-1.43		-2.39	-4.11	chelatase, putative
DVU0653	-0.45	-0.74		-2.00	-2.19	sigma-54 dependent transcriptional regulator, putative/response regulator
DVU0676	-0.74	-1.45		-1.83	-2.81	amino acid ABC transporter, permease protein, His/Glu/Gln/Arg/opine family
DVU0680	-0.11	-0.19		-2.04	-3.92	sensory box histidine kinase
DVU0707	-0.13	-0.23		-1.14	-2.00	TRAP dicarboxylate family transporter
DVU0722	-0.69	-1.07		-3.17	-5.43	response regulator
DVU0729	-0.65	-1.27		-1.49	-2.17	hypothetical protein
DVU0731	-0.10	-0.17		-1.97	-2.62	hypothetical protein
DVU0747	0.40	0.48		-1.78	-2.54	ABC transporter, ATP-binding protein
DVU0769	-0.28	-0.56		-1.67	-2.08	pyridoxal kinase, putative
DVU0786	-0.44	-0.62		-1.36	-2.20	penicillin-binding protein
DVU0807	-0.06	-0.09		-1.40	-2.20	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase
DVU0836	0.86	1.62		-1.23	-2.04	tRNA (guanine-N1)-methyltransferase
DVU0867	-0.14	-0.24		-1.97	-2.49	aromatic amino acid decarboxylase, putative
DVU0884	0.03	0.06		-1.62	-2.28	conserved hypothetical protein
DVU0897	0.02	0.03		-1.61	-2.52	RNA modification enzyme, MiaB-family
DVU0899	-0.31	-0.61		-1.20	-2.16	conserved hypothetical protein
DVU0934	0.09	0.15		-1.40	-2.10	hypothetical protein
DVU0961	-0.74	-1.38		-1.79	-2.61	conserved hypothetical protein
DVU0962	-0.46	-0.85		-2.18	-3.07	hypothetical protein
DVU0965	-0.57	-0.91		-1.38	-2.19	hypothetical protein
DVU0978	-0.49	-0.90		-1.99	-2.64	ABC transporter, periplasmic substrate-binding protein, putative
DVU0980	-0.52	-1.02		-2.92	-3.90	DAK2 domain protein
DVU0981	-0.89	-1.65		-1.77	-2.76	multiphosphoryl transfer protein, putative
DVU0990	-0.04	-0.08		-1.79	-2.09	endonuclease III, putative
DVU0991	-0.41	-0.63		-2.02	-2.33	conserved hypothetical protein
DVU0992	-1.10	-1.98		-1.81	-3.10	chemotaxis protein CheV
DVU1004	-0.48	-0.91		-2.19	-3.41	membrane protein, putative
DVU1025	-0.23	-0.45		-1.49	-2.63	uracil phosphoribosyltransferase
DVU1272	0.58	0.89		-1.93	-2.74	general secretion pathway protein E, putative
DVU1284	0.00	0.01		-1.50	-2.11	primosomal protein n
DVU1390	-0.85	-1.07		-1.44	-2.43	hypothetical protein
DVU1447	-0.64	-1.20		-1.63	-2.15	CgeB family protein
DVU1555	0.10	0.15		-2.29	-2.83	hypothetical protein
DVU1559	-0.12	-0.21		-1.48	-2.18	aldehyde oxidoreductase
DVU1568	-1.00	-1.87		-1.90	-3.19	ferritin
DVU1570	-0.46	-0.89		-1.96	-2.39	pyruvate ferredoxin oxidoreductase, beta subunit
DVU1588	0.10	0.17		-1.52	-2.51	hypoxanthine phosphoribosyltransferase
DVU1593	0.73	1.42		-2.12	-2.44	chemotaxis protein CheY
DVU1608	-0.52	-0.96		-1.85	-2.20	DNA ligase, NAD-dependent
DVU1687	-0.81	-1.36		-1.40	-2.01	glycosyl transferase, group 2 family protein
DVU1772	-0.13	-0.24		-2.05	-2.07	pyridine nucleotide-disulfide oxidoreductase
DVU1811	-1.32	-1.87		-1.50	-2.26	protoheme IX farnesyltransferase, putative
DVU1812	-1.09	-1.89		-1.66	-2.31	cytochrome c oxidase, subunit II, putative
DVU1878	0.09	0.16		-1.87	-2.11	threonine aldolase, low-specificity
DVU1879	0.24	0.44		-1.47	-2.40	glycosyl transferase, group 1 family protein
DVU1881	-1.08	-1.99		-1.45	-2.46	phoH family protein

DVU1912	-0.06	-0.12		-1.17	-2.03	conserved hypothetical protein TIGR00150
DVU1913	-0.39	-0.77		-2.36	-2.72	aspartate kinase, monofunctional class
DVU1946	-0.35	-0.69		-1.73	-2.08	pyruvate ferredoxin oxidoreductase, beta subunit, putative
DVU1991	-0.66	-1.08		-1.42	-2.49	hypothetical protein
DVU2012	-0.37	-0.65		-2.72	-4.47	hypothetical protein
DVU2037	-0.97	-1.90		-2.02	-3.32	cobS protein, putative
DVU2047	-0.25	-0.46		-1.66	-2.25	hypothetical protein
DVU2093	0.08	0.13		-1.66	-2.73	thiH protein
DVU2117	-0.50	-0.94		-1.45	-2.35	membrane protein, putative
DVU2119	-0.80	-1.31		-2.14	-3.70	type II/III secretion system protein
DVU2124	-0.18	-0.32		-2.47	-2.89	conserved hypothetical protein
DVU2251	0.42	0.84		-1.51	-2.67	DNA-binding protein
DVU2306	-1.02	-1.99		-1.55	-2.89	phosphate transporter family protein
DVU2345	-0.68	-1.31		-2.26	-2.22	hypothetical protein
DVU2364	-0.82	-1.48		-2.58	-3.89	aminotransferase, classes I and II
DVU2388	-0.64	-1.23		-1.98	-2.74	tolQ protein
DVU2431	-0.01	-0.02		-1.22	-2.01	hypothetical protein
DVU2449	-0.54	-0.82		-1.77	-2.49	S-adenosylmethionine synthetase
DVU2472	-0.84	-1.55		-1.24	-2.26	conserved hypothetical protein
DVU2478	-0.80	-1.42		-1.97	-2.65	phosphate ABC transporter, permease protein, putative
DVU2483	-0.56	-1.05		-1.97	-2.23	cytochrome c family protein
DVU2503	-1.02	-1.98		-1.45	-2.53	UDP-N-acetylmuramate--alanine ligase
DVU2544	0.01	0.02		-1.99	-2.96	iron-sulfur cluster-binding protein
DVU2568	-0.20	-0.31		-2.03	-2.67	peptidase, M20/M25/M40 family
DVU2585	-0.72	-1.38		-1.95	-2.98	methyl-accepting chemotaxis protein
DVU2620	-0.47	-0.88		-1.40	-2.40	conserved hypothetical protein
DVU2658	-0.09	-0.17		-1.75	-2.71	conserved hypothetical protein
DVU2673	-0.82	-1.64		-1.72	-2.15	anaerobic glycerol-3-phosphate dehydrogenase, subunit A, truncation
DVU2676	-0.95	-1.52		-3.30	-3.09	hypothetical protein
DVU2677	-0.02	-0.03		-1.72	-2.12	sensor histidine kinase/response regulator
DVU2699	-0.03	-0.06		-2.85	-4.07	transglycosylase SLT domain protein
DVU2735	-0.61	-1.01		-1.73	-2.10	phenylacetate-coenzyme A ligase
DVU2763	-0.18	-0.35		-1.87	-2.50	TPR/GGDEF domain protein
DVU2806	-0.98	-1.65		-1.51	-2.05	MotA/TolQ/ExbB proton channel family protein
DVU2807	-0.29	-0.56		-2.20	-2.45	biopolymer transport protein, ExbD/TolR family
DVU2851	-0.95	-1.73		-2.67	-4.42	tail protein, putative
DVU2853	-0.46	-0.89		-2.93	-4.24	phage baseplate assembly protein V, putative
DVU2857	-0.92	-1.79		-2.47	-3.63	conserved hypothetical protein
DVU2863	-1.11	-2.00		-4.14	-7.26	hypothetical protein
DVU2866	-1.09	-1.98		-2.79	-3.44	conserved hypothetical protein
DVU2897	-0.56	-0.97		-2.02	-2.18	conserved hypothetical protein
DVU2898	0.12	0.14		-1.86	-2.84	conserved hypothetical protein
DVU2901	0.10	0.19		-1.42	-2.14	aspartate carbamoyltransferase
DVU2970	-0.71	-1.02		-1.55	-2.04	acetyltransferase, GNAT family
DVU2983	1.01	1.86		-2.37	-2.23	3-isopropylmalate dehydratase, small subunit
DVU2984	0.98	1.87		-3.14	-3.46	conserved hypothetical protein
DVU2985	0.68	1.35		-1.54	-2.55	3-isopropylmalate dehydrogenase
DVU2996	-0.45	-0.72		-1.93	-3.09	NAD-dependent epimerase/dehydratase family protein
DVU2997	-0.31	-0.60		-2.04	-3.05	hypothetical protein
DVU3010	-0.80	-1.36		-1.53	-2.47	aminotransferase, DegT/DnrJ/EryC1/StrS family
DVU3018	-0.70	-1.25		-1.40	-2.11	radical SAM domain protein
DVU3055	-0.17	-0.30		-2.16	-2.12	ribonuclease, Rne/Rng family
DVU3068	-0.06	-0.12		-1.48	-2.09	GAF domain/sensory box/EAL domain protein
DVU3074	0.24	0.46		-1.66	-2.12	membrane protein, putative

DVU3099	-0.71	-1.40		-1.61	-2.13	tolQ protein
DVU3104	-0.27	-0.52		-1.70	-2.58	peptidoglycan-associated lipoprotein, putative
DVU3112	-0.18	-0.35		-1.60	-2.11	TPR domain protein
DVU3126	-0.67	-1.19		-1.54	-2.23	paraquat-inducible protein A, degenerate
DVU3131	-0.23	-0.45		-1.94	-3.12	transcriptional regulator, putative
DVU3133	-0.47	-0.91		-2.41	-2.40	glycerol uptake facilitator protein
DVU3134	-0.53	-0.93		-1.44	-2.21	glycerol kinase
DVU3196	0.08	0.15		-1.74	-2.89	twin-arginine translocation pathway signal sequence domain protein
DVU3230	-0.93	-1.72		-1.85	-2.37	flagellar synthesis regulator FleN
DVU3235	-1.06	-2.00		-1.18	-2.08	IMP cyclohydrolase, putative
DVU3294	-0.68	-1.32		-2.27	-2.45	aldehyde dehydrogenase (NADP) family protein
DVU3321	-0.43	-0.80		-2.25	-3.69	hypothetical protein
DVU3324	0.49	0.91		-1.83	-2.13	ABC transporter, ATP-binding protein
DVU3342	-0.10	-0.15		-1.21	-2.32	hypothetical protein
DVU3349	-0.80	-1.47		-1.43	-2.02	pyruvate flavodoxin/ferredoxin oxidoreductase, thiamine diP-binding domain protein
DVU3371	-0.95	-1.46		-3.06	-3.71	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase
DVUA0023	-0.79	-1.25		-2.29	-2.77	ABC transporter, permease protein, putative
DVUA0025	-1.74	-1.40		-2.02	-2.28	response regulator receiver domain protein
DVUA0061	-0.01	-0.02		-1.45	-2.42	membrane protein, putative
DVUA0138	-0.09	-0.14		-2.42	-2.67	sensor histidine kinase

Table S4 Up-regulated ORFs under salt shock but no significant changes under salt adaptation

	Salt shock			Salt adaptation		
Locus tag	Ratio	Zscore		Ratio	Zscore	Annotation
DVU0051	1.20	2.35		0.16	0.15	conserved hypothetical protein TIGR00044
DVU0052	1.09	2.06		0.68	1.11	GTP-binding protein Era
DVU0058	1.05	2.01		0.45	0.47	efflux transporter, RND family, MFP subunit
DVU0062	1.63	3.14		0.88	1.72	RND efflux system, outer membrane protein, NodT family
DVU0063	1.25	2.21		1.04	1.83	transcriptional regulator, MarR family
DVU0066	2.13	3.21		0.84	0.80	cytidine/deoxycytidylate deaminase domain protein
DVU0087	1.35	2.60		0.78	1.52	conserved domain protein
DVU0094	1.41	2.64		-0.06	-0.04	methyl-accepting chemotaxis protein
DVU0153	1.35	2.59		0.39	0.70	hypothetical protein
DVU0230	2.03	3.77		1.36	1.54	transcriptional regulator cII, putative
DVU0285	1.22	2.28		0.33	0.46	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit
DVU0368	1.77	2.57		-0.01	-0.01	hypothetical protein
DVU0371	1.74	3.43		0.72	1.14	conserved hypothetical protein
DVU0434	1.13	2.12		0.55	0.94	Ech hydrogenase, subunit EchA, putative
DVU0452	1.40	2.66		0.00	0.01	hypothetical protein
DVU0460	1.93	3.76		0.21	0.41	predicted phospho-2-dehydro-3-deoxyheptonate aldolase
DVU0461	2.08	3.95		0.06	0.10	predicted 3-dehydroquinate synthase
DVU0462	2.00	3.77		-0.54	-0.99	chorismate mutase/prephenate dehydratase
DVU0463	2.03	3.78		-0.24	-0.43	3-phosphoshikimate 1-carboxyvinyltransferase
DVU0464	1.29	2.52		-0.52	-0.66	prephenate dehydrogenase
DVU0465	1.80	3.46		-0.34	-0.27	anthranilate synthase, component I
DVU0466	1.53	2.99		0.85	1.37	anthranilate synthase, glutamine amidotransferase component
DVU0467	1.86	3.59		-0.10	-0.16	anthranilate phosphoribosyltransferase
DVU0468	1.64	3.23		0.40	0.66	indole-3-glycerol phosphate synthase
DVU0469	1.79	3.50		-0.16	-0.25	N-(5-phosphoribosyl)anthranilate isomerase
DVU0470	1.16	2.17		-0.01	-0.01	tryptophan synthase, beta subunit
DVU0471	1.41	2.73		0.09	0.15	tryptophan synthase, alpha subunit

DVU0477	1.99	3.22		1.07	1.83	isocitrate dehydrogenase, NADP-dependent
DVU0485	1.25	2.15		-1.24	-1.23	membrane protein, putative
DVU0503	1.35	2.60		-0.07	-0.09	polyribonucleotide nucleotidyltransferase
DVU0506	2.27	4.29		0.99	1.74	DHH family protein
DVU0507	1.61	2.85		0.34	0.47	conserved hypothetical protein
DVU0508	1.60	3.03		0.01	0.03	translation initiation factor IF-2
DVU0510	1.49	2.74		-0.17	-0.28	N utilization substance protein A
DVU0523	1.11	2.01		-0.18	-0.31	negative regulator of flagellin synthesis FlgM
DVU0530	1.93	3.07		2.20	1.77	response regulator, rrf1 protein
DVU0602	2.08	4.07		1.24	0.02	hypothetical protein
DVU0605	1.91	3.64		1.03	1.09	hypothetical protein
DVU0637	1.27	2.43		1.15	1.97	conserved hypothetical protein
DVU0745	1.49	2.76		0.08	0.15	ABC transporter, periplasmic substrate-binding protein
DVU0774	1.73	3.25		-0.02	-0.03	ATP synthase, F1 epsilon subunit
DVU0775	1.52	2.72		0.22	0.33	ATP synthase, F1 beta subunit
DVU0776	1.46	2.66		-0.46	-0.61	ATP synthase, F1 gamma subunit
DVU0777	1.72	2.94		-0.25	-0.33	ATP synthase, F1 alpha subunit
DVU0778	1.98	3.63		0.35	0.45	ATP synthase, F1 delta subunit
DVU0779	1.63	2.98		0.00	0.00	ATP synthase F0, B subunit, putative
DVU0780	1.55	3.05		0.48	0.84	ATP synthase F0, B subunit, putative
DVU0798	1.14	2.01		0.94	1.39	hypothetical protein
DVU0818	1.20	2.03		-1.53	-2.36	conserved domain protein
DVU0834	2.72	5.30		0.78	1.32	ribonuclease HII
DVU0845	1.26	2.42		0.26	0.36	hypothetical protein
DVU0849	1.20	2.12		-0.48	-0.60	heterodisulfide reductase, iron-sulfur-binding subunit, putative
DVU0871	1.56	2.76		0.64	1.11	uridylate kinase
DVU0872	1.47	2.69		0.57	1.00	glycosyl transferase, group 2 family protein
DVU0877	2.51	4.79		0.02	0.02	hypothetical protein
DVU0879	1.27	2.46		1.22	1.91	hypothetical protein
DVU0929	1.22	2.35		0.10	0.18	GTP-binding protein, GTP1/OBG family
DVU0930	1.49	2.74		0.46	0.71	glutamate 5-kinase
DVU0931	1.99	3.89		0.50	0.91	phosphomethylpyrimidine kinase
DVU0933	1.53	2.95		-0.19	-0.13	response regulator
DVU1009	1.67	2.79		0.85	1.55	hypothetical protein
DVU1030	1.29	2.55		1.17	1.90	universal stress protein family
DVU1079	2.47	4.70		1.62	1.39	tRNA modification GTPase TrmE
DVU1080	1.77	3.40		1.35	1.32	iron-sulfur cluster-binding protein
DVU1138	1.09	2.13		1.33	1.67	hypothetical protein
DVU1207	1.33	2.39		-0.23	-0.32	3-oxoacyl-(acyl-carrier-protein) synthase III
DVU1231	1.08	2.07		0.27	0.38	ammonium transporter
DVU1248	1.05	2.02		0.39	0.73	arginyl-tRNA synthetase
DVU1274	1.71	3.14		-1.00	-1.69	hypothetical protein
DVU1300	1.26	2.20		0.73	1.14	translation elongation factor G
DVU1301	1.16	2.03		-0.22	-0.39	hypothetical protein
DVU1306	1.34	2.49		-0.41	-0.53	ribosomal protein L2
DVU1307	1.54	2.62		-0.64	-0.96	ribosomal protein S19
DVU1310	1.19	2.25		-0.69	-1.05	ribosomal protein L16
DVU1311	1.21	2.27		-0.71	-1.01	ribosomal protein L29
DVU1313	1.33	2.60		-0.66	-1.04	ribosomal protein L14
DVU1315	1.46	2.70		-0.58	-0.90	ribosomal protein L5
DVU1316	1.31	2.49		-0.66	-1.02	ribosomal protein S14
DVU1317	1.12	2.17		-0.36	-0.49	ribosomal protein S8
DVU1318	1.10	2.14		-0.09	-0.14	ribosomal protein L6
DVU1319	1.19	2.18		-0.55	-0.91	ribosomal protein L18
DVU1320	1.53	2.68		-0.54	-0.95	ribosomal protein S5

DVU1321	1.56	2.93		-0.40	-0.75	ribosomal protein L30
DVU1322	1.57	2.94		-0.47	-0.88	ribosomal protein L15
DVU1323	2.10	3.74		-0.29	-0.54	preprotein translocase, SecY subunit
DVU1330	1.09	2.14		0.58	1.08	ribosomal protein L17
DVU1331	1.10	2.00		0.12	0.23	transcriptional regulator, LysR family
DVU1333	1.78	2.92		-0.04	-0.08	hypothetical protein
DVU1480	1.58	2.75		0.75	1.24	conserved domain protein
DVU1491	2.05	4.01		1.36	1.79	conserved hypothetical protein
DVU1494	1.22	2.26		0.44	0.72	hypothetical protein
DVU1506	2.14	3.95		1.03	1.29	hypothetical protein
DVU1507	2.03	3.75		-0.53	-0.52	hypothetical protein
DVU1573	1.48	2.60		1.10	1.74	peptidyl-tRNA hydrolase
DVU1619	1.08	2.05		0.48	0.75	phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent
DVU1621	1.31	2.50		-1.14	-1.31	hypothetical protein
DVU1636	1.46	2.60		0.37	0.71	inorganic pyrophosphatase, manganese-dependent
DVU1641	1.91	3.70		1.75	1.84	conserved hypothetical protein
DVU1644	1.76	2.96		1.19	1.89	permease, putative
DVU1659	1.05	2.09		0.59	0.62	hypothetical protein
DVU1664	1.13	2.18		0.96	1.80	GTP-binding protein
DVU1665	1.07	2.10		0.50	0.89	3-dehydroquinate dehydratase, type II
DVU1666	1.08	2.05		-0.47	-0.81	translation elongation factor P
DVU1675	1.23	2.26		0.99	1.72	hypothetical protein
DVU1694	2.71	4.76		1.51	1.33	C4-type zinc finger protein, DksA/TraR family
DVU1695	1.44	2.84		0.05	0.06	tail fiber assembly protein, putative
DVU1697	1.54	2.92		0.84	0.79	hypothetical protein
DVU1698	1.41	2.60		1.54	1.87	hypothetical protein
DVU1726	1.83	3.42		-0.41	-0.39	hypothetical protein
DVU1728	2.05	4.04		1.45	1.51	conserved hypothetical protein
DVU1755	1.12	2.19		1.52	1.91	hypothetical protein
DVU1764	1.12	2.22		1.01	1.93	conserved hypothetical protein
DVU1791	1.09	2.10		0.35	0.63	GatB/Yqey family protein
DVU1821	1.20	2.20		0.22	0.17	conserved hypothetical protein
DVU1823	1.35	2.37		0.20	0.16	glutamate synthase, iron-sulfur cluster-binding subunit, putative
DVU1952	1.23	2.32		0.76	1.39	hypothetical protein
DVU1969	1.18	2.28		0.95	0.99	hypothetical protein
DVU1970	2.15	3.61		1.74	1.13	response regulator
DVU2184	1.14	2.10		0.96	0.73	DNA-binding domain, excisionase family
DVU2197	1.75	3.19		0.77	0.80	site-specific recombinase, phage integrase family
DVU2206	1.53	2.83		0.33	0.54	conserved hypothetical protein
DVU2275	1.49	2.55		1.02	1.90	sigma-54 dependent transcriptional regulator
DVU2298	1.43	2.74		0.82	1.42	glycine/betaine/L-proline ABC transporter, permease protein
DVU2299	1.48	2.56		0.87	1.49	glycine/betaine/L-proline ABC transporter, ATP binding protein
DVU2343	1.63	2.74		1.37	1.32	amino acid ABC transporter, ATP-binding protein
DVU2433	1.89	3.55		0.74	0.55	hypothetical protein
DVU2434	1.84	3.19		0.52	0.79	hypothetical protein
DVU2591	1.05	2.01		-0.65	-0.72	tail fiber assembly protein, putative
DVU2649	1.13	2.02		1.77	1.86	hypothetical protein
DVU2672	1.48	2.93		0.99	1.72	membrane protein, putative
DVU2816	2.44	3.60		1.81	1.39	multidrug resistance protein
DVU2914	1.20	2.18		-0.04	-0.08	peptide chain release factor 1
DVU2915	1.83	3.52		1.29	1.33	hypothetical protein
DVU2922	1.18	2.05		0.06	0.11	preprotein translocase, SecE subunit
DVU2928	1.10	2.02		-0.46	-0.77	DNA-directed RNA polymerase, beta subunit
DVU2954	1.23	2.18		0.36	0.61	GGDEF domain protein
DVU2981	1.36	2.51		-0.18	-0.18	2-isopropylmalate synthase

DVU3090	1.46	2.86		0.08	0.13	outer membrane protein, OMPP1/FadL/TodX family
DVU3092	1.37	2.57		0.30	0.30	hypothetical protein
DVU3149	1.15	2.23		0.52	0.94	signal peptide peptidase SppA, 36K type
DVU3188	1.08	2.09		0.60	1.02	NLP/P60 family protein
DVU3203	1.11	2.15		0.29	0.44	DNA polymerase III, delta prime subunit, putative
DVU3212	1.21	2.27		1.14	1.68	pyridine nucleotide-disulfide oxidoreductase
DVU3245	1.44	2.66		0.61	1.19	transcription elongation factor GreA
DVU3290	1.48	2.62		1.11	1.05	conserved domain protein
DVU3291	1.54	2.31		0.30	0.48	glutamate synthase, iron-sulfur cluster-binding subunit, putative
DVU3292	2.09	3.92		-0.85	-1.17	pyridine nucleotide-disulfide oxidoreductase
DVU3300	1.45	2.78		0.39	0.49	hypothetical protein
DVU3308	1.28	2.45		-0.12	-0.20	metallo-beta-lactamase family protein
DVU3310	1.64	3.18		0.11	0.14	ATP-dependent RNA helicase, DEAD/DEAH family
DVU3326	2.41	4.55		1.67	1.42	multidrug resistance protein, Smr family
DVU3385	2.34	4.28		1.59	1.30	hypothetical protein
DVU3394	1.32	2.35		1.25	1.40	hypothetical protein
DVUA0089	1.78	2.88		-1.10	-1.14	hypothetical protein

Table S5 Down-regulated ORFs under salt shock but no significant changes under salt adaptation

	Salt shock			Salt adaptation		
Locus tag	Ratio	Zscore		Ratio	Zscore	Annotation
DVU0006	-1.33	-2.34		-0.67	-0.99	universal stress protein family
DVU0019	-1.12	-2.23		-0.49	-0.83	nigerythrin
DVU0041	-1.12	-2.16		-0.98	-1.31	transglycosylase, SLT family
DVU0080	-1.44	-2.79		-2.19	-1.75	fumarate hydratase, class II
DVU0118	-1.30	-2.17		-1.08	-1.19	sigma-54 dependent transcriptional regulator/response regulator
DVU0123	-2.34	-3.85		-0.87	-0.49	membrane protein, putative
DVU0138	-1.25	-2.27		-0.86	-1.48	response regulator
DVU0200	-1.27	-2.30		-1.78	-1.74	major head protein
DVU0211	-1.61	-2.15		-0.98	-1.80	tail tube protein, putative
DVU0259	-1.87	-3.65		-1.11	-1.97	DNA-binding response regulator
DVU0280	-1.44	-2.53		-0.94	-1.69	glycosyl transferase, group 1 family protein
DVU0337	-1.18	-2.18		-1.62	-1.91	hypothetical protein
DVU0344	-1.25	-2.43		0.95	1.36	methyl-accepting chemotaxis protein
DVU0428	-1.16	-2.07		-0.44	-0.64	conserved hypothetical protein
DVU0521	-1.09	-2.05		-0.42	-0.70	carbon storage regulator
DVU0522	-1.32	-2.39		-0.07	-0.09	conserved hypothetical protein
DVU0624	-1.43	-2.82		-0.45	-0.76	NapC/NirT cytochrome c family protein
DVU0630	-1.20	-2.23		-0.05	-0.09	hypothetical protein
DVU0699	-1.32	-2.38		-2.29	-1.83	sensory box sensor histidine kinase/response regulator, authentic frameshift
DVU0817	-1.45	-2.81		-0.91	-1.59	hypothetical protein
DVU0881	-1.83	-3.24		-0.28	-0.49	translation elongation factor G, putative
DVU0964	-1.23	-2.33		-0.25	0.00	Glu/Leu/Phe/Val dehydrogenase family protein
DVU0987	-1.45	-2.81		-1.02	-1.86	heavy metal-binding domain protein
DVU1021	-1.13	-2.08		-0.11	-0.17	conserved hypothetical protein
DVU1049	-1.09	-2.09		-0.33	-0.59	ABC transporter, ATP-binding protein
DVU1093	-1.31	-2.52		-0.78	-1.23	HAD-superfamily hydrolase, subfamily IA, variant 3
DVU1238	-1.54	-2.92		-0.90	-1.55	amino acid ABC transporter, periplasmic amino acid-binding protein
DVU1375	-1.16	-2.05		-0.57	-1.00	hypothetical protein
DVU1458	-1.21	-2.37		-0.99	-1.64	chemotaxis protein CheZ, putative
DVU1541	-1.12	-2.17		0.28	0.35	hypothetical protein
DVU1544	-1.21	-2.36		-0.05	-0.08	mechanosensitive ion channel family protein
DVU1548	-1.33	-2.44		0.07	0.09	outer membrane transport protein, OmpP1/FadL/TodX family

DVU1837	-1.17	-2.24		-0.43	-0.80	competence protein, putative
DVU1838	-1.25	-2.31		-0.75	-1.40	thioredoxin reductase
DVU1882	-1.74	-2.90		-1.48	-1.56	HDIG domain protein
DVU1922	-1.24	-2.31		-0.63	-0.80	periplasmic [NiFe] hydrogenase, large subunit, isozyme 1
DVU1935	-1.35	-2.54		-1.65	-1.43	phosphonate ABC transporter, permease protein
DVU1948	-1.13	-2.18		-0.25	-0.38	conserved hypothetical protein
DVU2016	-1.11	-2.03		-0.01	-0.01	GGDEF domain protein
DVU2019	-1.27	-2.36		-0.65	-0.87	conserved hypothetical protein
DVU2108	-1.83	-3.42		-1.22	-1.79	MTH1175-like domain family protein
DVU2109	-1.81	-2.77		-0.84	-1.42	MTH1175-like domain family protein
DVU2162	-1.78	-2.83		-1.72	-1.44	hypothetical protein
DVU2312	-1.23	-2.38		-0.71	-0.90	hypothetical protein
DVU2313	-1.25	-2.44		-1.47	-1.52	6-phosphogluconolactonase
DVU2386	-1.49	-2.72		0.18	0.18	ABC transporter, permease protein
DVU2398	-1.20	-2.20		-0.60	-0.98	conserved hypothetical protein
DVU2399	-2.03	-3.70		-0.91	-1.39	hydrogenase, putative
DVU2400	-1.81	-3.14		-0.92	-1.39	hydrogenase, putative
DVU2401	-1.56	-2.82		-0.36	-0.63	hydrogenase, iron-sulfur cluster-binding subunit, putative
DVU2402	-1.80	-3.40		-1.00	-1.62	heterodisulfide reductase, A subunit
DVU2403	-1.35	-2.56		-0.85	-1.25	heterodisulfide reductase, B subunit
DVU2404	-1.13	-2.12		-0.60	-0.96	heterodisulfide reductase, C subunit
DVU2445	-1.25	-2.01		-1.07	-1.85	hypothetical protein
DVU2451	-1.36	-2.54		0.06	0.12	L-lactate permease family protein
DVU2452	-1.89	-3.71		0.20	0.29	hypothetical protein
DVU2502	-1.20	-2.34		-0.23	-0.28	UDP-N-acetylenolpyruvoylglucosamine reductase
DVU2579	-1.45	-2.83		-1.24	-0.02	TPR domain protein
DVU2609	-1.36	-2.65		-0.55	-0.96	chemotaxis MotB protein, putative
DVU2615	-1.50	-2.87		-2.02	-1.93	bacterial extracellular solute-binding protein, family 3
DVU2626	-1.24	-2.28		-1.46	-1.61	hypothetical protein
DVU2778	-1.25	-2.14		-1.48	-1.64	hypothetical protein
DVU2782	-1.41	-2.42		-0.55	-0.77	hypothetical protein
DVU2791	-1.31	-2.51		-0.62	-1.18	cytochrome c family protein
DVU2794	-1.34	-2.48		-1.41	-1.28	electron transport complex protein RnfG, putative
DVU2860	-1.18	-2.15		1.33	1.13	conserved hypothetical protein
DVU2861	-1.24	-2.40		-0.20	-0.30	hypothetical protein
DVU2935	-1.19	-2.25		-0.68	-1.27	phosphoglycerate mutase
DVU3009	-1.21	-2.36		-0.67	-1.21	radical SAM domain protein
DVU3012	-1.17	-2.24		-0.79	-1.15	membrane protein, putative
DVU3015	-1.59	-2.91		-1.15	-1.23	conserved domain protein
DVU3024	-1.35	-2.62		-1.50	-1.89	hypothetical protein
DVU3025	-1.34	-2.47		-1.50	-1.53	pyruvate-ferredoxin oxidoreductase
DVU3026	-1.93	-3.72		-1.41	-1.99	L-lactate permease family protein
DVU3033	-1.33	-2.26		-1.88	-1.95	iron-sulfur cluster-binding protein
DVU3187	-1.97	-3.75		-1.10	-1.90	DNA-binding protein HU
DVU3220	-1.31	-2.54		-1.04	-1.90	sigma-54 dependent transcriptional regulator/response regulator
DVU3231	-1.06	-2.06		-1.43	-1.69	flagellar biosynthesis protein FlhF, putative
DVU3263	-1.22	-2.34		-1.49	-1.55	fumarate reductase, iron-sulfur protein
DVU3271	-1.28	-2.46		0.10	0.14	cytochrome d ubiquinol oxidase, subunit I
DVU3293	-1.12	-2.21		-1.55	-1.88	thiamine pyrophosphate-requiring enzyme
DVU3319	-1.13	-2.06		-1.37	-1.26	proline dehydrogenase/delta-1-pyrroline-5-carboxylate dehydrogenase
DVU3350	-1.16	-2.17		-1.03	-1.88	iron-sulfur cluster-binding protein
DVU3355	-1.46	-2.65		-1.02	-1.12	SPFH domain/Band 7 family protein
DVU3356	-1.66	-3.15		-0.49	-0.90	NAD-dependent epimerase/dehydratase family protein
DVUA0070	-1.72	-2.13		0.17	0.28	conserved domain protein

DVUA0098	-2.02	-3.05		0.36	0.55	dehydrogenase, putative
DVUA0099	-2.09	-3.14		-0.93	-1.12	HAMP domain protein
DVUA0100	-1.98	-3.66		-0.44	-0.62	sigma-54 dependent transcriptional regulator
DVUA0104	-1.83	-3.25		0.72	0.73	type III secretion inner membrane protein, HrcV family
DVUA0114	-2.35	-2.44		-1.03	-1.32	hypothetical protein
DVUA0115	-2.35	-3.22		-1.26	-0.99	type III secretion system protein, YscF family
DVUA0116	-2.90	-3.27		-1.32	-1.47	conserved hypothetical protein
DVUA0119	-1.77	-2.78		-1.71	-1.95	type III secretion system ATPase
DVUA0121	-1.84	-2.16		-0.72	-0.66	type III secretion system protein, YopQ family
DVUA0123	-1.69	-2.50		-1.83	-1.53	anti-anti-sigma factor
DVUA0124	-2.51	-4.17		-1.36	-1.18	sigma factor serine-protein kinase
DVUA0125	-1.15	-2.02		1.02	0.84	transglycosylase, SLT family
DVUA0135	-1.70	-2.18		0.73	1.32	CRISPR-associated protein Cas2

Table S6 Up-regulated ORFs under both salt shock and salt adaptation conditions

	Salt shock			Salt adaptation		
Locus tag	Ratio	Zscore		Ratio	Zscore	Annotation
DVU0061	1.38	2.44		1.80	3.24	multidrug resistance protein, putative
DVU0065	3.29	6.39		3.31	5.06	hypothetical protein
DVU0085	1.44	2.74		2.48	3.72	tryptophan synthase, beta subunit
DVU0223	2.88	5.32		2.83	4.68	conserved hypothetical protein
DVU0224	2.50	4.36		1.78	2.92	conserved hypothetical protein
DVU0273	1.25	2.20		1.69	2.93	conserved hypothetical protein
DVU0308	2.68	4.43		2.84	4.07	membrane protein, putative
DVU0367	1.38	2.69		2.02	2.87	Ser/Thr protein phosphatase family protein
DVU0369	2.52	4.41		2.30	2.35	hypothetical protein
DVU0458	1.42	2.74		1.93	3.27	hypothetical protein
DVU0504	1.36	2.55		1.20	2.16	ribosomal protein S15
DVU0525	1.24	2.30		2.99	2.76	transcriptional regulator, MarR family
DVU0526	1.81	3.39		3.01	5.05	drug resistance transporter, putative
DVU0529	2.59	3.67		3.51	2.98	transcriptional regulator, rrf2 protein, putative
DVU0531	2.17	3.71		3.75	2.62	hmc operon protein 6
DVU0533	1.52	2.55		3.45	2.75	hmc operon protein 4
DVU0535	2.04	2.98		2.64	2.92	hmc operon protein 2
DVU0536	2.37	4.23		3.00	2.75	high-molecular-weight cytochrome C
DVU0603	2.50	4.67		1.57	2.92	hypothetical protein
DVU0604	1.48	2.80		2.49	4.10	hypothetical protein
DVU0878	3.01	5.77		1.74	2.59	dnaK suppressor protein, putative
DVU1277	1.24	2.29		2.21	3.91	hypothetical protein
DVU1294	1.48	2.11		1.34	2.54	conserved hypothetical protein
DVU1334	1.33	2.57		1.11	2.01	trigger factor
DVU1505	2.44	4.71		1.47	2.04	holin, putative
DVU1508	1.97	3.92		2.47	3.66	conserved hypothetical protein
DVU1645	2.46	4.70		1.98	3.20	transcriptional regulator, ArsR family
DVU1689	1.82	3.44		1.76	2.41	hypothetical protein
DVU1696	1.72	3.14		2.38	3.61	hypothetical protein
DVU1729	2.75	4.76		2.14	2.13	killer protein, putative
DVU1730	2.28	3.91		2.46	3.36	DNA-binding protein
DVU1760	1.33	2.49		1.67	2.32	transcriptional regulator, TetR family
DVU1855	1.53	2.98		2.89	2.79	integrase, truncation
DVU1872	1.83	3.50		1.62	2.12	hypothetical protein
DVU1971	3.65	6.16		3.25	5.53	conserved domain protein
DVU2002	1.15	2.01		2.22	3.99	hypothetical protein
DVU2073	3.66	6.11		3.08	4.28	chemotaxis protein CheY

DVU2176	1.16	2.28		1.71	2.69	hypothetical protein
DVU2274	1.48	2.56		1.40	2.03	hypothetical protein
DVU2279	1.64	2.37		1.80	2.97	hypothetical protein
DVU2280	1.90	3.72		2.34	3.04	amino acid permease family protein
DVU2284	1.33	2.44		1.36	2.46	hypothetical protein
DVU2294	2.45	4.65		2.43	3.19	femAB family protein
DVU2297	1.62	3.13		1.59	3.11	glycine/betaine/L-proline ABC transporter, periplasmic-binding protein
DVU2527	1.40	2.33		1.15	2.08	transcriptional regulator, putative
DVU2571	1.88	3.54		2.87	5.09	ferrous iron transport protein B
DVU2572	1.98	3.60		2.11	3.21	ferrous iron transport protein A, putative
DVU2574	1.46	2.31		1.21	2.13	ferrous ion transport protein, putative
DVU2634	1.49	2.64		2.32	3.67	hypothetical protein
DVU2647	1.70	3.16		1.49	2.37	endoribonuclease, L-PSP family
DVU2651	1.33	2.50		2.51	3.74	hypothetical protein
DVU2652	3.22	6.23		2.13	3.91	hypothetical protein
DVU2653	1.18	2.13		3.11	5.66	hypothetical protein
DVU2744	1.78	3.20		2.40	4.48	high-affinity branched-chain amino acid ABC transporter, perisplasmic amino acid binding protein
DVU2815	2.46	4.75		2.89	2.53	outer membrane efflux protein
DVU2817	3.71	7.00		3.69	3.39	multidrug resistance protein
DVU2818	4.40	8.00		5.77	7.61	hypothetical protein
DVU2819	1.47	2.86		1.61	2.24	transcriptional regulator, TetR family
DVU2822	1.29	2.38		2.87	3.43	TRAP dicarboxylate family transporter
DVU2918	1.73	3.27		2.31	2.39	hypothetical protein
DVU2941	1.95	3.10		1.21	2.13	conserved hypothetical protein
DVU2956	1.55	2.74		1.39	2.40	sigma-54 dependent transcriptional regulator
DVU2987	1.15	2.22		2.90	5.42	hypothetical protein
DVU2988	1.33	2.21		3.51	6.67	phage shock protein A
DVU2989	2.17	3.94		2.96	2.87	psp operon transcriptional activator
DVU3302	1.56	2.42		5.42	3.82	hypothetical protein
DVU3327	1.29	2.42		2.21	3.94	multidrug resistance protein, Smr family
DVU3330	1.09	2.02		1.74	3.00	conserved hypothetical protein
DVU3331	1.23	2.21		2.65	3.78	hypothetical protein
DVUA0002	2.03	2.68		2.23	2.34	ParA family protein
DVUA0082	2.22	3.41		1.97	2.55	site-specific recombinase, phage integrase family
DVUA0084	1.82	2.01		3.48	3.48	transcriptional regulator, AbrB family

Table S7 Down-regulated ORFs under both salt shock and salt adaptation conditions

	Salt shock			Salt adaptation		
Locus tag	Ratio	Zscore		Ratio	Zscore	Annotation
DVU0131	-1.13	-2.22		-2.93	-3.20	hypothetical protein
DVU0132	-1.03	-2.03		-2.16	-4.02	membrane protein, putative
DVU0197	-1.42	-2.41		-1.26	-2.01	phage portal protein, lambda family
DVU0198	-1.36	-2.33		-2.30	-3.07	minor capsid protein C, degenerate
DVU0199	-1.66	-2.98		-3.13	-4.22	conserved hypothetical protein
DVU0203	-1.20	-2.27		-2.64	-2.40	conserved hypothetical protein
DVU0204	-1.38	-2.45		-2.15	-2.43	lipoprotein, putative
DVU0205	-1.49	-2.61		-2.35	-3.32	hypothetical protein
DVU0206	-1.04	-2.06		-1.98	-3.04	hypothetical protein
DVU0207	-1.33	-2.55		-1.60	-2.36	hypothetical protein
DVU0208	-1.48	-2.65		-2.21	-2.23	hypothetical protein
DVU0209	-1.50	-2.82		-1.79	-3.18	conserved hypothetical protein
DVU0210	-1.24	-2.30		-2.20	-4.12	tail sheath protein, putative
DVU0219	-1.43	-2.76		-2.38	-4.36	tail protein, putative

DVU0253	-1.21	-2.00		-1.82	-3.02	oxidoreductase, FAD/iron-sulfur cluster-binding domain protein
DVU0299	-2.70	-5.14		-2.73	-3.95	anaerobic ribonucleoside-triphosphate reductase, putative
DVU0300	-2.39	-4.60		-2.68	-4.62	radical SAM domain protein
DVU0598	-1.52	-2.78		-3.90	-4.99	carbon starvation protein A, putative
DVU0599	-1.27	-2.24		-3.46	-6.39	carbon starvation protein A, putative
DVU0608	-1.52	-2.80		-1.47	-2.32	methyl-accepting chemotaxis protein
DVU0646	-1.08	-2.10		-1.92	-2.51	precorrin-2 C20-methyltransferase
DVU0743	-1.40	-2.10		-2.43	-3.43	sensory box histidine kinase
DVU1378	-1.19	-2.21		-1.58	-2.55	ketol-acid reductoisomerase
DVU1411	-1.50	-2.79		-1.39	-2.58	thiamine biosynthesis protein ThiC
DVU1778	-2.00	-3.89		-1.53	-2.68	cation efflux family protein
DVU2034	-1.11	-2.01		-1.46	-2.12	hypothetical protein
DVU2036	-1.24	-2.28		-2.37	-2.91	helix-turn-helix protein, CopG family
DVU2305	-1.07	-2.06		-2.38	-2.68	conserved hypothetical protein
DVU2349	-1.41	-2.60		-1.89	-2.80	carbohydrate phosphorylase family protein
DVU2514	-1.43	-2.72		-2.65	-3.08	pyruvate kinase
DVU2515	-1.31	-2.51		-1.93	-2.32	HD domain protein
DVU2543	-1.51	-2.96		-1.86	-2.29	hybrid cluster protein
DVU2569	-1.15	-2.21		-1.41	-2.05	peptidyl-prolyl cis-trans isomerase, FKBP-type
DVU2798	-1.71	-3.24		-1.84	-2.56	ApbE family protein
DVU2858	-1.27	-2.04		-2.43	-4.36	tail tube protein, putative
DVU2859	-1.21	-2.08		-1.30	-2.10	tail sheath protein, putative
DVU2862	-1.79	-3.43		-3.19	-5.07	hypothetical protein
DVU2864	-1.17	-2.15		-1.25	-2.16	hypothetical protein
DVU2867	-1.39	-2.41		-3.98	-6.18	holin
DVU2869	-1.36	-2.60		-2.64	-4.43	major head protein
DVU2870	-1.65	-2.77		-2.40	-4.24	conserved hypothetical protein
DVU2931	-1.11	-2.13		-2.53	-3.86	sensory box histidine kinase
DVU3027	-1.41	-2.51		-2.10	-2.29	glycolate oxidase, subunit GlcD
DVU3029	-1.77	-3.33		-2.47	-2.56	phosphate acetyltransferase
DVU3030	-2.30	-4.11		-1.89	-2.32	acetate kinase
DVU3031	-2.08	-3.96		-2.28	-3.64	conserved hypothetical protein
DVU3032	-1.86	-3.24		-2.07	-2.40	conserved hypothetical protein
DVU3045	-1.27	-2.26		-2.84	-3.86	sensory box histidine kinase/response regulator
DVU3107	-1.33	-2.33		-1.99	-3.51	cytochrome c family protein
DVU3113	-1.24	-2.41		-1.69	-3.07	carbamoyl-phosphate synthase, small subunit
DVU3183	-1.33	-2.15		-1.28	-2.35	desulfoferrodoxin
DVU3184	-1.84	-2.95		-1.13	-2.15	rubredoxin
DVU3221	-1.40	-2.71		-1.45	-2.07	sensor histidine kinase