Global Transcriptome Analysis of the Heat Shock Response of *Shewanella oneidensis*

Running Title: Genomic Analysis of *S. oneidensis* Heat Shock Response

Haichun Gao[1], Sarah Wang[2], Xueduan Liu[1], Tinfeng Yan[1], Liyou Wu[1], Eric Alm[2], Adam Arkin[2], Dorothea K. Thompson[1], and Jizhong Zhou[1]*

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831[1]; *Departments of Bioengineering and Chemistry, University of California, Berkeley, Berkeley, CA 94720*[2]

*Corresponding Author: Jizhong Zhou, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831. Tel: 865-576-7544, Fax: 865-576-8646, E-mail: zhouj@ornl.gov.

ABSTRACT

Shewanella oneidensis **is an important model organism for bioremediation studies because of its diverse respiratory capabilities. However, the genetic basis and regulatory mechanisms underlying the ability of *S. oneidensis* to survive and adapt to various environmentally relevant stresses is poorly understood. To define this organism's molecular response to elevated growth temperatures, temporal gene expression profiles were examined in cells subjected to heat stress using whole-genome DNA microarrays for *S. oneidensis* MR-1. Approximately 15% (711) of the predicted *S. oneidensis* genes represented on the microarray were significantly up- or down-regulated ($P < 0.05$) over a 25-min period following shift to the heat shock temperature (42°C). As expected, the majority of *S. oneidensis* genes exhibiting homology to known chaperones and heat shock proteins (Hsps) were highly and transiently induced. In addition, a number of predicted genes encoding enzymes in glycolysis and the pentose cycle, [NiFe] dehydrogense, serine proteases, transcriptional regulators (MerR, LysR, and TetR families), histidine kinases, and hypothetical proteins were induced in response to heat stress. Genes encoding membrane proteins were differentially expressed, suggesting that cells possibly alter their membrane composition or structure in response to variations in growth temperature. A substantial number of the genes encoding ribosomal proteins displayed down-regulated co-expression patterns in response to heat stress, as did genes encoding prophage and flagellar proteins. Finally, based on computational comparative analysis of the upstream promoter regions of *S. oneidensis* heat-**

inducible genes, a putative regulatory motif, showing high conservation to the *Escherichia coli* σ^{32}-binding consensus sequence, was identified.

Shewanella oneidensis MR-1 (formerly *Shewanella putrefaciens* strain MR-1), a facultatively anaerobic γ-proteobacterium, possesses remarkably diverse respiratory capacities and is widely distributed in nature, with aquatic environments and sediments as its primary habitats (40). In addition to utilizing oxygen as a terminal electron acceptor during aerobic respiration, *S. oneidensis* can anaerobically respire various organic and inorganic substrates, including fumarate, nitrate, nitrite, thiosulfate, elemental sulfur, trimethylamine *N*-oxide (TMAO), dimethyl sulfoxide (DMSO), Fe (III), Mn (III) and (IV), Cr (VI), and U (VI). The metal ion-reducing capabilities of this bacterium may possibly be exploited for the bioremediation of metal contaminants in the environment. However, application of such organisms of bioremediation potential to contaminated sites is often complicated by unpredictable microbial interactions and various environmental stresses at the given site. In addition, little is known about the molecular basis underlying a microorganism's perturbation response behavior or the impact of environmental stresses (e.g., temperature upshift, pH fluctuations, and nutrient limitation) on its ability to reduce metals and radionuclides.

Variation in growth temperature is a common stress encountered in nature. The heat shock response, which is elicited by a sudden increase in optimal growth temperature, has been widely used as a model system for studying the impact of stress on biological systems (27). The hallmark of this adaptive cellular response is the induction of a limited set of proteins, termed as heat shock proteins (Hsps) or more generally,

molecular chaperones. The spectrum of Hsps synthesized in different organisms after a stress challenge displays notable similarities (26, 29, 47). Several Hsp families can be distinguished and are designated according to their average apparent molecular mass, e.g., Hsp100, Hsp90, Hsp70 (DnaK), Hsp60 (GroEL) and small Hsps (2, 3, 11, 13, 24, 29, 41). In addition, ATP-dependent proteases such as ClpP and Lon are known to be Hsps (12). In general, heat shock proteins or chaperones play roles in protein folding, protein degradation, assembly of protein complexes, and transport of proteins across membranes.

DNA microarrays have already been used to characterize the transcriptome of bacteria responding to different growth conditions and environmental stresses (7, 9, 17, 18, 23, 30, 33, 34, 36). In the present study, we used DNA microarrays covering ~99% of the total predicted protein-encoding open reading frames (ORFs) in *S. oneidensis* to investigate differential gene expression profiles in response to a temperature upshift from 30 to 42°C over a period of 25 min. Microarray analysis revealed that *S. oneidensis* homologues of known heat shock proteins, together with genes not previously demonstrated to be affected by heat stress and those unique to this metal-reducing bacterium, showed significant differential expression in response to temperature upshift. Based on the gene expression data and computational analysis, a putative consensus sequence for *S. oneidensis* heat shock gene promoters was derived that closely resembles the *E. coli* σ^{32} recognition site.

Whole genome microarray construction. PCR primers for 4,648 of 4931 predicted ORFs on both the *S. oneidensis* chromosome and plasmid (excluding 43 unique and 240

multicopy genes) were designed using PRIMEGENS (6) and then synthesized by MWG Biotech (Highpoint. NC.). The following criteria were used to identify optimal forward and reverse primers to generate PCR products specific to each of the selected ORFs: (i) The entire ORF was used as a probe if it was less than 75% similar to all other genes in the genome; (ii) for homologous genes, the maximal portion of the genes showing less than 75% similarity were selected as a specific probe; (iii) for homologous genes where no specific fragments could be identified, one of the genes was selected as a probe to represent the gene group; and (iv) each primer contained 20-25 oligonucleotides. To simplify the PCR amplifications, most of the primer sets were designed to have annealing temperatures of approximately 65ºC based on Primer 3 (Whitehead Institute).

ORF-specific fragments were amplified using the following cycling conditions: 30- sec denaturation at 95°C, 1-min annealing at 60°C, and 1.5-min extension at 72°C along with an initial 5-min denaturation at 95°C and a final extension reaction at 72°C for 7 min. All PCR products were purified using the QIAquick 96-well purification kit (Qiagen, Valencia, CA). The quality of the amplified products was checked by 1.5% agarose gel electrophoresis and ethidium bromide staining. Amplified DNA fragments were considered correct if PCR reactions contained a single product of the expected size. PCR reactions of 451 genes consistently failed to yield satisfactory products (e.g., no product, product of the wrong size, multiple bands, or faint bands), even after re-designed primers were used in the reaction. Of the 4648 total predicted genes, 4197 ORFs were correctly amplified, representing approximately 90% of the genome. We used specific 50-mer oligonucleotides to represent the 451 ORFs not successfully amplified by PCR. In total, the PCR amplicons and oligo probes represented approximately 99% of the total

predicted gene content of *S. oneidensis* MR-1. Probes were printed onto SuperAmine slides (Telechem, Inc., Sunnyvale, Calif.) as described previously (37).

Sampling, microarray experiments, and data analysis. *S. oneidensis* DSP10, a spontaneous rifampin-resistant derivative of *S. oneidensis* MR-1 that is used for mutant generation, was used in this study (37). For all experiments, a single colony of DSP10 was used to inoculate 1 ml of Luria-Bertani (LB, Difco, Detroit, Mich.) in 12 ml plastic tubes and grown overnight at 30°C (optimal growth temperature) on a rotary platform (200 rpm). This culture was then used to inoculate 50 ml of medium pre-warmed to 30°C at an OD_{600} of 0.01. The flask was shaken on a rotary platform (250 rpm) until a mid-log OD_{600} of 0.60 was attained. Samples (zero time) were taken from the 50-ml culture, and a 25-ml aliquot was transferred to a 250-ml flask pre-warmed to 42°C and incubated in a 42°C water bath shaker. A parallel identical experiment was performed with cultures subjected to the non-shock temperature (30°C). Preliminary experiments were carried out to determine proper heat shock conditions with samples at 5, 15, 25, 40 and 60 min at either 37°C or 42°C. Samples were removed from cultures grown at 30°C and 42°C at 5, 15, and 25 min and centrifuged for 10 s at the maximum speed in a 5415R centrifuge (Eppendorf, Germany). The culture supernatant was removed immediately, and the tubes containing the cell pellet were placed in liquid nitrogen. Cell density changed only slightly after the 25-min heat shock period ($OD_{600,}$ 0.60-0.66 on average). RNA isolation, cDNA labeling, hybridization and microarray scanning were performed as described previously (37).

To determine signal fluorescence intensities for each spot, 16-bit TIFF scanned images were analyzed using the software ImaGene version 5.5 (Biodiscovery, Marina Del Rey, CA). Any spot that had < 75% of pixels and >2 standard deviation above the local background in both channels was excluded from further analysis. The resulting data files were normalized and further analyzed using GeneSpring version 5.1 (Silicon Genetics, Redwood City, Ca.).

Assessment of Array Data Quality. The reliability of the microarray data generated was assessed by using co-hybridization of two cDNA samples both prepared from a total RNA sample. The pattern of hybridization revealed a linear correlation with no more than a 2-fold change in the relative expression level of 99.7% of the genes (data not shown). This control experiment suggested that genes with an expression ratio beyond this range were either down- or up-regulated. Therefore, only genes identified as being up- or down-regulated by an expression ratio of at least 2-fold were chosen for further analysis, even though a 1.5-fold cutoff has recently been reported as being biologically significant (19, 33, 34).

To validate the microarray results, eight ORFs were selected for real-time quantitative reverse transcription-PCR (RT-PCR) analysis with the same RNA samples used in the array hybridizations based on the level and reproducibility of changes observed in the microarray experiments. Primers were designed using Omiga software (Oxford Molecular Ltd., San Diego, CA) and were synthesized by Applied Biosystems. Primer sequences are listed in Table 1.

PCR products amplified from these ORFs were single-band fragments of 99-101 bp in length, as confirmed by agarose gel electrophoresis. A 100-bp fragment of the *dnaK* gene, which was amplified by PCR with genomic DNA as the template, was used to construct the standard curve. The reaction was performed with 50 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C and monitored in an iCycler iQ real-time PCR detection system (Bio-Rad, Hercules, CA). A standard curve was derived from PCR products representing each ORF with genomic DNA as the template and used to convert threshold crossings to log copy numbers. The expression of each gene was determined from three replicates on a single real-time RT-PCR experiment. The expression ratio was recorded as the fold difference in quantity of real-time RT-PCR product from samples grown at the treatment versus control temperature. A high level of concordance was observed between the microarray and real-time RT-PCR data despite quantitative differences in the level of change (Fig. 1). On the average, real-time RT-PCR expression values were 2.5-fold higher than those obtained by microarray hybridization, thus suggesting that microarray analysis may underestimate the change in gene expression. This underestimation of fold changes by DNA microarray analysis has been previously reported (17, 34, 44).

In addition to real-time RT-PCR analysis, expression differences for gene pairs within the same predicted operon or gene pairs selected at random were compared to determine whether changes in gene expression were experimentally significant (Fig. 2). Adjacent genes were considered to comprise a single transcriptional unit if (i) both genes occurred on the same strand, and (ii) the two genes were separated by an intergenic distance of < 55 bp, or orthologs of the two genes occurred within 5000 bp of each other in multiple, distantly related species. This simple definition correctly classified about

88% of experimentally studied adjacent gene pairs into "same" or "different" operon pairs (data not shown) according to the EcoCyc database (21). Deviations from the Gaussian distribution were determined using the Kolmogorov-Smirnov test (22).

Consistent with our expectation, we observed that genes within the same operon responded more similarly to heat shock conditions than did genes randomly selected from the genome. As a quantitative measure of co-expression within a single experiment, we used the magnitude of the difference in log ratio expression levels for the two genes. Genes that are up- or down-regulated to the same extent should have a difference near zero. Because only a limited number of genes significantly change their expression level in any given experiment, it is likely that a large number of unrelated transcriptional units, which are not differentially expressed, will have a small log-ratio difference. Therefore, we limited our analyses to gene pairs in which at least one gene was in the 250 most over-expressed or the 250 most under-expressed gene list. Gene pairs within the same operon were constructed by exhaustively enumerating all such pairs, and random pairs were chosen from the genome resulting in a sample size of 5000.

As shown in Figure 2, the within-operon pairs showed much smaller log-ratio differences than did gene pairs chosen at random, thus confirming the high quality of the expression data particularly for the significantly over- or under-expressed genes. To demonstrate that the underlying distributions of log-ratio differences for the samples of gene pairs within operons and those selected at random are significantly different, the Kolmogorov-Smirnov test was performed for data from the three time points (5 min, $D = 0.4128$, p-value < 2.2e-16; 15 min, $D = 0.398$, p-value < 2.2e-16; 25 min, $D = 0.348$, p-

value < 2.2e-16). Highly significant *p*-values were obtained even without the requirement that one gene be significantly over- or under-expressed.

Genomic Response of *S. oneidensis* to Heat Stress. Whole-genome DNA microarrays were used to obtain a comprehensive, general description of the molecular response mounted by *S. oneidensis* when challenged by heat stress. In total, 609 genes (323/286 induced/repressed) at 5 min, 711 genes (358/353) at 15 min, and 466 genes (240/226) at 25 min exhibited significant ($p < 0.05$) differential expression at a 2-fold or greater level in at least four of the six replicates in response to a temperature upshift from 30°C to 42°C. These total gene numbers represent about 13% (5 min), 16% (15 min), and 10% (25 min) of the 4,648 ORFs represented on the array. Figure 3 summarizes the overall genomic response of *S. oneidensis* to temperature upshift by grouping the differentially expressed genes into their functional role categories, as assigned based on TIGR's annotation of the MR-1 genome sequence (15; http://www.tigr.org/).

The wide distribution of putative functional roles attributed to the differentially expressed genes indicates the extent of the molecular response that enables *S. oneidensis* cells to survive and eventually adapt to thermal stress. As shown in Figure 3, a large number of the genes that were down-regulated in response to heat shock had annotated functions in energy metabolism (bar 7), whereas most of the genes related to protein fate (bar 10) that showed differential expression were induced, as opposed to repressed, upon temperature upshift. For genes of known function, those encoding proteins involved in cellular processes (bar 4), energy metabolism (bar 7), protein fate (bar 10), regulatory functions (bar 13), and substrate transport (bar 16) were among the most up-regulated

genes in response to heat stress. Most notably, many of the genes whose expression was altered by the temperature increase encode proteins of unknown function (bar 18), thus suggesting a more extensive heat shock stimulon than what can be deduced from the sequence annotation. Along with genes involved in energy metabolism, functionally undefined genes (bar 18) were among the most down-regulated genes in response to heat shock.

In contrast to steady-state or single-time-point studies, time course experiments are particularly valuable in providing insight into the mechanism regulating a bacterial response to stress and provide useful data for generating computational models of stress response pathways. Our temporal gene expression analysis indicated that the global changes in mRNA expression levels upon temperature increase were largely transient. *S. oneidensis* cells responded with significant changes ($p < 0.05$, ≥ 2 fold) in the expression level of selected genes during the first 15 min and then the percentage of such genes decreased about 20% at 25 min. Data from our preliminary experiments showed that less than 20% of these genes remained differentially expressed at 1 h (data not shown). These gene expression profiles suggest that *S. oneidensis* appears to rapidly readjust its transcript levels to a new steady state at the heat shock temperature (42°C), thereby allowing the bacterium to survive the stress. This is in agreement with previous findings reported for both *E. coli* and *Campylobacter jejuni* (30, 34).

Hierarchical clustering of temporal gene expression data. To identify co-regulated patterns of gene expression, Genesis software (Version 1.2.1; Graz University of Technology [http://genome.tugraz.at]) was used to perform hierarchical clustering of the

filtered genes that varied significantly ($p < 0.05$ and a fold change ≥ 2) in their expression profiles in response to temperature upshift from 30 to 42°C. Eight clusters were observed that represented specific patterns of regulation (Fig. 4).

Cluster A comprised 27 genes that showed a constant level of induction (approximately 2- to 4-fold) over the 25-min heat shock period (Fig. 4). Genes encoding hypothetical proteins (SO0886, 1443, 2542, 2911, 3274, 3377, 3381, 3512, 3585, 3682, 3765, 4593) accounted for 45% of the differentially expressed genes grouped in this cluster. The majority of the remaining genes code for proteins involved in energy metabolism (*aceA, aceB, cfa, edd, gapA-2, pgl,* SO1471, 3683). The mechanism underlying the involvement of isocitrate lyase and malate synthase A, encoded by *aceA* and *aceB* respectively, in the heat shock response of *S. oneidensis* is presently unknown. These proteins work together to short-circuit the tricarboxylic acid (TCA) cycle, therefore rendering most of the TCA components unnecessary. One possible explanation is that *S. oneidensis* cells challenged with heat stress, unlike those growing under normal physiological conditions, may divert usage of the protein synthesis machinery from purposes of rapid growth to producing proteins important or critical for survival and cell maintenance. The shock response redirects energy expenditure and protein synthesis toward the rapid overproduction of Hsps, which enable cell survival.

Interestingly, several genes encoding enzymes of the glycolytic pathway were induced upon heat treatment. Based on the sequence annotation, S. *oneidensis* does not appear to possess a complete glycolytic pathway; a homologue for the third enzyme, phosphofructokinase, appears to be missing (15). Genome sequence analysis suggests

that the bacterium may rescue glycolysis via the pentose phosphate pathway by employing glyceraldehyde-3-phosphate dehydrogenase (encoded by *gapA-2*), which catalyzes the only reaction coupled with NADH production in glycolysis. Because central carbon pathways are interconnected, excesses or deficiencies in one pathway should impact others (42). It might be possible that cells increase the rate of glycolysis, thereby restoring the intracellular ATP level, especially while the TCA cycle is shutdown or operating at a lower level of activity. Consistent with this supposition is the observation that expression of *pykA*, which encodes pyruvate kinase (another glycolytic enzyme), was induced in response to heat shock. Pyruvate kinase catalyzes a step coupled with substrate-level phosphorylation. Additionally, genes from a predicted operon consisting of *zwf*, *pgl*, and *edd* were highly induced. The products of these genes catalyze the key steps connecting the glycolytic pathway to the pentose phosphate pathway, thus enabling the bacterium to bypass the missing phsophofructokinase step in glycolysis. These steps also produce NADPH, which may be needed for other cellular processes or used to generate ATP by oxidative phosphorylation. Interestingly, two highly induced enzymes AdhE and GabD (aldehyde-alcohol dehydrogenase and succinate-semialdehyde dehydrogenase respectively), are also involved in the catalytic processes of generating NADH or NADPH, thus suggesting that *S. oneidensis* could utilize these pathways to ensure the availability of electron carriers during the heat shock response. Independent confirmation of *aceB* and *zwf* expression by real-time RT-PCR showed over a 20-fold induction.

Cluster B included 220 genes that exhibited variable expression levels during the 25-min heat shock period. Most of the genes in this cluster were up-regulated about 2- to

4-fold during at least one of the three time points but at other time points induction in expression was not significant or even repressed. Cluster B was dominated by genes encoding hypothetical proteins (108/220). The remaining genes included a subset of genes encoding proteins known or presumed to be involved in chemotaxis and ion transport. This subset of genes included *cheA, cheB, cheR-1, cheW, cheY, motA, motB* (encode chemotaxis proteins); and *ktrA, ktrB, nhaA, nosF, nosy, pstB-2, pstC,* SO0534, 2045, 2865, 3333 3690, 3768, 3801, 3802, 4598 (encode ion transport proteins). This observation is in agreement with the findings of Richmond et al. (30), who demonstrated that the expression of chemotaxis and ion transport genes in *E. coli* was induced in response to heat shock. Cluster B also included genes encoding transcriptional regulators belonging to MerR, LysR, and TetR families. Expression of these genes was increased slightly early in the heat shock response and exhibited higher expression levels at 25 min. *S. oneidensis* has a relatively small repertoire of regulatory genes compared with *Vibrio cholerae*, which is phylogenetically closely related to *S. oneidensis*. This repertoire includes 57 response regulators and 88 two-component regulatory system proteins, which could allow rapid detection and response to environmental changes (15, 16). Our microarray data revealed that 46 of these regulatory genes were up-regulated, and an additional 17 members grouped in other clusters showed a similar expression pattern. It is not clear how these regulators are involved in the heat shock response and further investigation is needed.

Cluster C contained 111 genes. The expression of these genes was immediately increased up to at least 5-fold following temperature upshift. After 15 min of exposure to 42°C, their expression decreased over time. Genes encoding hypothetical proteins

dominate this cluster at the level of 50%. Genes of known function in cluster C encode proteins involved in energy metabolism such as Ni/Fe hydrogenase (*hyaB, hydC*), formate dehydrogenase (*fdhB, SO4509*), and anaerobic reductase (*dmaA-2, dmsB-1, nrdD, nrdG*). The *hyaB* and *hydC* genes encode a classic [NiFe] hydrogenase, an important component in anaerobic respiratory electron transport systems. It is unclear whether the transcriptional induction of these genes, whose products are involved in anaerobic respiration, is elicited by heat stress.

Cluster D consisted of 51 genes. The gene expression patterns in this cluster resembled those of cluster C. However, the initial mRNA expression levels were induced up to 16-fold, followed by a more gradual decline in expression. The major group of proteins encoded by genes in this cluster have predicted cellular functions related to protein fate and primarily included chaperones, chaperonins, and heat shock proteins. Sequence annotation of the *S. oneidensis* MR-1 genome revealed at least 22 homologues of chaperones/chaperonins and heat shock proteins, several of which have been well-characterized in other bacteria and include DnaK, DnaJ, GroEL, GroES, GrpE, HtpG, and Lon/La proteases. Also identified in the MR-1 genome are genes predicted to encode known regulators of the heat shock response, namely σ^{32} and σ^{E}. In *Escherichia coli*, the induction of the majority of Hsps results from a rapid and transient increase in the cellular level of the alternative 32-kDa sigma subunit (σ^{32}), encoded by *rpoH*, which complexes with the core RNA polymerase (RNAP) and directs the RNAP holoenzymes to transcribe specifically from heat-regulated promoters (14, 26, 43, 46, 47), thus permitting both steady-state and stress-induced levels of Hsp expression (5, 48). The increase in the intracellular concentration of the σ^{32} transcription factor is due to a

concomitant increase in both the stability and synthesis of σ^{32}. In addition, alternative σ factors, σ^{E} (σ^{24}) and σ^{54}, encoded by *rpoE* and *rpoN* respectively, are involved in regulation of certain subsets of Hsps. σ^{E} is essential for transcription from one (*rpoHp*$_3$) of the promoters of *rpoH* and the promoter of *htrA* encoding a periplasmic endopeptidase essential for growth at high temperature, whereas σ^{54} plays roles in regulation of α-heat shock proteins (25). Table 2 shows the temporal expression levels for some of the predicted *S. oneidensis* heat shock proteins. While the magnitude of induction for each of these genes varied, they displayed a maximal fold change at an early stage in the response with a decrease in induction over time. This is consistent with the notion that the rapid cellular accumulation of Hsps upon a temperature upshift is followed by an adaptation period, during which the levels of Hsps are readjusted to the new steady-state growth conditions at the higher temperature (30). In our study, σ^{32}, encoded by *rpoH*, had a relatively lower induction at the early stage and kept the same level for the first 15 min following temperature upshift (Table 2). This finding differs somewhat from the observation in *E. coli* that the increase in *rpoH* mRNA synthesis (less than 2-fold) following heat shock appears to contribute negligibly to the overall σ^{32} induction (8, 35). Independent confirmation of expression of heat shock genes *dnaK* and *rpoH* using real-time RT-PCR displayed induction folds that were up to 3-fold higher than the microarray results.

Cluster E included 9 genes whose expression was constant after temperature upshift. All of these genes showed strong transcriptional induction (at least 8-fold). Four of these genes encode hypothetical proteins (SO0543, 1094, 1442, 4359) and clustered

with genes encoding metabolic enzymes (*pflB*, *dmaA2*, *phrB*), as well as proteins with annotated functions in cellular processes (SO1158, *mexE*).

Cluster G comprised more than 223 genes that showed transient reductions in expression after temperature upshift. These down-regulated genes primarily included those encoding hypothetical proteins (30%), proteins involved in metabolic pathways, translation or DNA replication (60%), and proteins involved in regulation (6%). The most notable subgroup of genes displaying transcriptional repression included 37 out of 52 ribosomal structural genes (*rpl*, *rpm*, and *rps* operons). While, in several gene expression studies with *Saccharomyces cerevisiae* and *Campylobacter jejuni*, it has been observed that the expression level of ribosomal genes is similarly affected by heat stress, as well as alkylating agents (10, 20, 34). This observation may provide insight into the mechanism of ribosomes as sensors of heat shock (39), although several lines of evidence support the proposal that the free pool of DnaK and DnaJ may serve as a cellular thermometer, monitoring changes in cellular concentration of unfolded or denatured proteins (28, 38). Expression of ribosomal genes in *S. oneidensis* was repressed mostly at the early stage of heat shock and nearly returned to a basal level 25 min after the temperature upshift. Such a pattern suggests a brief initial growth arrest, during which the cell redirects/re-channels its energy usage to the increased expression of genes encoding proteins involved in the protective response to heat stress.

Clusters F and H were comprised of 41 and 59 genes, respectively, which exhibited decreased mRNA expression at all time points after the temperature was elevated to 42°C. The difference resides in the level of repression. Expression of genes in

cluster F decreased as much as 20 fold with an average reduction of 3.5 fold, whereas genes grouped in cluster H largely exhibited decreases less than 3 fold. Major members of these clusters are genes coding for hypothetical proteins and metabolic pathway components. An interesting finding was the observation that a number of genes from cluster H showing decreased expression encode proteins belonging to prophage families. *S. oneidensis* has three prophages, two of which are phylogenetically distinct phages related to the *E. coli* Mu (MuSo1 and MuSo2) and a lambda-like phage (LambdaSo). Most of genes (67%, 12/18) encoding LambdaSo proteins were down-regulated more than 2-fold, while about 33% (3/9) and 43% (6/14) of the genes for MuSo1 and MuSo2 proteins, respectively, displayed at least 2-fold repression. Finally, another subset of functionally defined genes from cluster F encoded flagellar proteins and shared a similar expression pattern as that of the prophage genes. The nature of these prophage and flagellar proteins in the heat shock response is presently unknown.

Hypothetical proteins. More than 41% of the *S. oneidensis* ORFs encode hypothetical proteins. In our study, genes for hypothetical proteins make up to 38% (3 time- point average) of 2-fold above changes in both up- and down-regulated gene expression. As for possible operons containing multiple genes encoding hypothetical proteins, we observed co-upregulation of all members from four such operons: *SO1442/SO1443*, *SO2861/SO2862/SO2863*, *SO3764/SO3765/SO3766*, and *SO4260/SO4261* (Table 3). The consistency of expression of these genes under the heat shock condition provides basic evidence to support such operon structure. Unfortunately, little is known about these proteins, although the protein encoded by *SO3765* may be a member of PspA that suppresses sigma54-dependent transcription. Two genes (*SO1094, SO3386*) displayed the

highest induction through the entire heat-shock period. The SO1094 protein may be a GreA-like transcription elongation factor that enables continuation of RNA transcription past template-encoded arresting sites, whereas the SO3386 protein belongs to a family of uncharacterized bacterial proteins. Interestingly, *SO1264* and *SO1274*, undoubtedly from different operons, encode possible members of a beta subunit family of sarcosine oxidase, suggesting that sarcosine oxidase may have a role in heat shock response.

Computational Prediction of σ^{32}-Binding Consensus Motif in *S. oneidensis*. To predict genes regulated by the σ^{32} transcription factor, we used AlignACE (31) to search for potential regulatory motifs upstream of heat-inducible genes, as identified by microarray analysis, and compared the derived *S. oneidensis* consensus sequence with that of the *E. coli* σ^{32}-binding motif. In the upstream regions of heat shock genes, there exists a sequence that is nearly identical to the consensus sequence derived from *E. coli* heat shock promoters. Based on a comparison of these promoters from a couple of bacteria including *E. coli* and *V. cholerae*, we propose a *S. oneidensis* consensus sequence for σ^{32}-controlled promoters having T-n-n-T-n-n-C-n-C-T-T-G-A-A-A in the -35 region and C-C-C-C-A-T-n-T-a in the -10 region with 13-15 bp separating the two elements (Fig. 5). Together with the induction of annotated heat shock genes at the elevated temperature, these observations suggest that the heat shock response in *S. oneidensis* is mediated by a similar mechanism as in *E. coli*.

Sequence Blast analysis revealed an extremely high similarity (85% positives) between *rrmJ* of *S. oneidesis*, encoding the ribosomal RNA large subunit methyltransferase J, and *fts*J of *E. coli*, indicating that the *rrmJ* operon, as in *E. coli* (4), is

a heat shock operon. A hypothetical protein, encoded by *SO2017*, is very likely under the control of σ^{32}. The protein contains a domain closely related to thioredoxin, suggesting that it may participate in redox reactions. While protein alignment analysis confirmed it as a thioredoxin domain-containing protein that may be involved in posttranslational modification and protein turnover, little information has been obtained by using Prospect protein structure prediction software (1, 45).

In addition to σ^{32}, σ^{24}, encoded by *rpoE*, has been reported to be involved in the regulation of the heat shock response in *E. coli*. The promoter sequence recognized by the *S. oneidensis* σ^{24} is not known because of insufficient knowledge. Our data showed that changes in expression of *rpoE* were in the range of natural experimental variation, suggesting that σ^{24} may only play a minor role in the heat shock response of *S. oneidensis* or its gene may be induced at a higher temperature as in *E. coli*.

Conclusion. The primary objective of this study was to characterize the transcriptome of *S. oneidensis* in response to heat stress using whole-genome DNA microarrays to monitor temporal gene expression. Over 10% of transcriptionally active genes displayed at least a two-fold induction upon temperature upshift. The expression patterns of 8 of these genes were independently confirmed using real-time RT-PCR. In general, the heat shock response in *S. oneidensis* is similar to that in *E. coli*. Moreover, the identified consensus sequences for heat shock gene promoters in both bacteria are virtually identical. However, it is noteworthy that the hypothetical protein SO2017, likely under the control of σ^{32}, shows no similarity to any protein known thus far. Our analysis illustrated the value and utility of microarray expression profiling in defining bacterial stimulons and

regulons. Our future work will focus on inactivating genetic elements such as sigma factors and other transcriptional regulators that might control critical stress responses in *S. oneidensis*.

ACKNOWLEDGMENTS

This research was supported by The United States Department of Energy under the Genomes To Life and Microbial Genome Programs of the Office of Biological and Environmental Research, Office of Science. Oak Ridge National Laboratory is managed by University of Tennessee-Battelle LLC for the Department of Energy under contract DE-AC05-00OR22725.

Figure legends:

FIG. 1. Comparison of expression measurements by microarray and real time RT-PCR assays. The fold changes in gene expression in response to temperature upshift from 30 to 42°C were log transformed (in base 2). Numbers 5, 15, and 25 represent the time points. The r values represent the concordance rate.

FIG. 2. Histogram of log ratio expression difference of gene pairs within the same operon vs. gene pairs selected at random. The normalized frequency was plotted against the ratio expression difference between the treatments and control.

FIG. 3. Differentially expressed genes grouped by functional classification according to the TIGR *S. oneidensis* genome database (provide web site). Columns: 1, Amino acid biosynthesis; 2, Biosythesis of cofactors, prosthetic groups, and carriers; 3, Cell envelope; 4. Cellular processed; 5, central intermediary metabolism; 6, DNA metabolism; 7, Energy metabolism; 8, fatty acid and phospholipid metabolism; 9, other categories; 10, protein fate; 11, protein synthesis; 12, purines, pyrimidines, nucleosides, and nucleotides; 13, regulatory functions; 14, signal transduction; 15, transcription; 16, transport and binding proteins; 17, unknown function, 18 hypothetical proteins.

FIG. 4. Hierarchical clustering of selected genes that varied significantly ($P < 0.05$ and a fold change > 2 at least at one time point) in their expression profiles in response to a temperature change from 30 to 42°C. Red color represents the levels of induction, while green color represents repression. Each row represents a single gene expression, and each column represents an individual time point after the temperature increase.

Fig.5. Consensus sequence for σ^{32} promoters. Upstream sequences of overexpressed genes in both *S. oneidensis* and *E. coli* were analyzed with AlignACE to find potential regulatory motifs. (A). A motif conserved in the upstream regions of up-regulated *S.*

oneidensis genes is nearly identical to the E. coli σ^{32} binding site. The sequence logo was prepared using public software at http://ep.ebi.ac.uk/EP/SEQLOGO/ (32). (B) Listed are promoters which display at least 7/12 matches in highlighted base pairs (bp) and 13-15 bp spacing. Stars (*) represent the positions with at least 50% matches.

REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman**. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389-3402.
2. **Buchner, J.** 1999. Hsp90 & Co.—a holding for folding. Trends Biochem. Sci. **24:**136-141.
3. **Bukau, B., E. Deuerling, C. Pfund, and E. A. Craig.** 2000. Getting newly synthesized proteins into shape. Cell **101:**119-122.
4. **Caldas, T., E. Binet, P. Bouloc, A. Costa, J. Desgres, and G. Richarme.** 2000. The **FtsJ**/RrmJ heat shock protein of *Escherichia* ***coli*** is a 23 S ribosomal RNA methyltransferase. J. Biol. Chem. **275:**16414-16419.
5. **Cowing, D. W., J. C. Bardwell, E. A. Craig, C. Woolford, R. W. Hendrix, and C. A. Gross.** 1985. Consensus sequence for *Escherichia coli* heat-shock promoters. Proc. Natl. Sci. Sci. USA **82:**2679-2683.

6. **Dong Xu, Guangshan Li, Liyou Wu, Jizhong Zhou, and Ying Xu.** 2002 PRIMEGENS: robust and efficient design of gene-specific probes for microarray analysis. Bioinformatics. **18:**1432-1437.
7. **Eisen, M. B., and P. O. Brown.** 1999. DNA arrays for analysis of gene expression. Methods Enzymol. **303:**179-205.
8. **Erickson, J., V. Vaughn, W.A. Walter, F.C. Neidhardt and C.A. Gross.** 1987. Regulation of the promoters and transcripts of *rpoH*, the *Escherichia coli* heat shock regulatory gene. Genes Dev. **1:** 419-432.
9. **Fisher, M. A., B. B. Plikaytis, and T. M. Shinnick.** 2002. Microarray analysis of the *Mycobacterium tuberculosis* transcriptional response to the acidic conditions found in phagosomes. J. Bacteriol. **184:**4025-4032.
10. **Gasch, A. P., P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen, G. Storz, D. Botstein, and P. O. Brown.** 2000. Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell **11:**4241-4257.
11. **Goloubinoff, P., A. Mogk, A. Peres Ben Zvi, T. Tomoyasu, and B. Bukau.** 1999. Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. Proc. Natl. Acad. Sci. USA **96:** 13732-13737.
12. **Gottesman, S.** 1996. Proteases and their targets in *Escherichia coli.* Annu. Rev. Genet. **30:**465-506.

13. **Grimaud, R., M. Kessel, F. Beuron, A. C. Steven, and M. R. Maurizi.** 1998. Enzymatic and structural similarities between the *Escherichia coli* ATP-dependent proteases, ClpXP and ClpAP. J. Biol. Chem. **273:**12476-12481.
14. **Gross, C. A.** 1996. Function and regulation of the heat shock proteins, p. 1382-1399. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, and ed. American Society for Microbiology, Washington, D.C.
15. **Heidelberg, J., I. Paulsen, K. Nealson, E. Gaidos, W. Nelson, T. Read, et al.** 2002. Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. Nat. Biotechnol. **20:**1118-1123.
16. **Heidelberg, J. F., J. A. Eisen, W. C. Nelson, R. A. Clayton, M. L. Gwinn, R. J. Dodson, D. H. Haft, E. K. Hickey, J. D. Peterson, et al.** 2000. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. Nature **406:**477-483.
17. **Helmann, J. D., M. F. Wu, P. A. Kobel, F. J. Gamo, M. Wilson, M. M. Morshedi, M. Navre, and C. Paddon.** 2001. Global transcriptional response of *Bacillus subtilis* to heat shock. J. Bacteriol. **183:**7318-7328.
18. **Hihara, Y., A. Kamei, M. Kanehisa, A. Kaplan, and M. Ikeuchi.** 2001. DNA micro-array analysis of cyanobacterial gene expression during acclimation to high light. Plant Cell **13:**793-806.
19. **Hughes, T. R., M. J. Marton, A. R. Jones, C. J. Roberts, R. Stoughton, C. D. Armour, H. A. Bennett, E. Coffey, H. Dai, Y. D. He, M. J. Kidd, A. M. King, M. R. Meyer, D. Slade, P. Y. Lum, S. B. Stepaniants, D. D. Shoemaker, D.**

Gachotte, K. Chakraburtty, J. Simon, M. Bard, and S. H. Friend. 2000. Functional discovery via a compendium of expression profiles. Cell **102:**109-126.

20. **Jelinsky, S. A., and L. D. Samson.** 1999. Global response of *Saccharomyces cerevisiae* to an alkylating agent. Proc. Natl. Acad. Sci. USA **96:**1486-1491.
21. **Karp, P. D., M. Riley, M. Saier, I. T. Paulsen, J. Collado-Vides, S. M. Paley, A. Pellegrini-Toole, C. Bonavides, and S. Gama-Castro.** 2002. The EcoCyc Database. Nucleic Acids Res. **30:**56-58.
22. **Khamis, H. J.** 2000. The two-stage delta-corrected Kolmogorov-Smirnov test, J. Appl. Stat. **27:** 439-450.
23. **Khodursky, A., B. Peter, N. Cozzerelli, D. Botstein, P. Brown, and C. Yanofsky.** 2000. DNA microarray analysis of gene expression in response to physiological and genetic changes that affect tryptophan metabolism in Escherichia coli. Proc. Natl. Acad. Sci. USA **97:**12170-12175.
24. **Kim, K. I., G. W. Cheong, S. C. Park, J. S. Ha, K. M. Woo, S. J. Choi, and C. H. Chung.** 2000. Heptameric ring structure of the heat-shock protein ClpB, a protein-activated ATPase in *Escherichia coli*. J. Mol. Biol. **303:**655-666.
25. **Kuczynska-Wisnik, D., E. Laskowska, and A. Taylor.** 2001. Transcription of *ibpB* heat shock gene is under control of σ^{32}- and σ^{54}-promoters, a third regulon of heat-shock response. Biochem. Biophys. Res. Commun. **284:**57-64.
26. **Lindquist, S., and E. A. Craig.** 1988. The heat-shock proteins. Annu. Rev. Genet. **22:**631-677.

27. **Mager, W. H. and A. J. J. De Kruijff.** 1995. Stress-induced transcriptional activation. Microbiol. Rev. **59:**506-531.
28. **Macarty, J. S., and G. C. Walker.** 1991. DnaK as a thermometer: threonine 199 is site of aurophosphorylation and is critical for ATPase activity. Proc. Natl. Acad. Sci. USA **88:**9513-9517.
29. **Narberhaus, F.** 2002. α-Crystallin-Type Heat Shock Proteins: Socializing Minichaperones in the Context of a Multichaperone Network. Microbiol. Mol. Biol. Rev. **66:**64-93.
30. **Richmond, C. S., J. D. Glasner, R. Mau, H. Jin, and F. R. Blattner.** 1999. Genome-wide expression profiling in *Escherichia coli* K-12. Nucleic Acids Res. **27:**3821-3835.
31. **Roth, F. P., J. D. Hughes, P. W. Estep, and G. M. Church.** 1998. Finding DNA regulatory motifs within unaligned noncoding sequences clustered by whole-genome mRNA quantitation. Nat. Biotechnol. **16:**939-945.
32. **Schneider, T. D., and R. M. Stephens.** 1990. Sequence logos: a new way to display consensus sequences. Nucleic Acids Res. **18:**6097-6100.
33. **Smoot, L. M., J. C. Smoot, M. R. Graham, G. A. Somerville, D. E. Sturdevant, C. A. Migliaccio, G. L. Sylva, and J. M. Musser.** 2001. Global differential gene expression in response to growth temperature alteration in group A *Streptococcus*. Proc. Natl. Acad. Sci. USA **98:**10416-10421.
34. **Stintzi, A.** 2003. Gene expression profile of *Campylobacter jejuni* in response to growth temperature variation. J. Bacteriol. **185:**2009-2016.

35. **Straus, D.B., W.A. Walter and C.A. Gross.** 1987. The heat shock response of *E. coli* is regulated by changes in the concentration of sigma32. Nature. **329:** 348-351.

36. **Tao, H., C. Bausch, C. Richmond, F. Blattner, and T. Conway.** 1999. Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. J. Bacteriol. **181:**6425-6440.

37. **Thompson, D. K., A. Beliaev, C. S. Giometti, D. P. Lies, K. H. Nealson, H. Lim, J. Yates, III, J. Tiedje, and J. Zhou.** 2002. Transcription and proteomic analysis of a ferric uptake regulator (Fur) mutant of *Shewanella oneidensis*: possible involvement of Fur in energy metabolism, regulation, and oxidative stress. Appl. Environ. Microbiol. **68:**881-892.

38. **Tomoyasu, T.**, **T. Ogura, T. Tatsuta, and B. Bukau.** 1998. Levels of DnaK and DnaJ provide tight control of heat shock gene expression and protein repair in *Escherichia coli*. Mol. Microbiol. **30:**567-581.

39. **VanBogelen, R. A., and F. C. Neidhardt.** 1990. Ribosomes as sensors of heat and cold shock in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**5589-5593.

40. **Venkateswaran, K., D. P. Moser, M. E. Dollhopf, D. P. Lies, D. A. Saffarini, B. J. MacGregor, D. B. Ringelberg, D. C. White, M. Nishijima, H. Sano, J. Burghardt, E. Stackebrandt, and K. H. Nealson.** 1999. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. Int. J. Syst. Bacteriol. **49:**705-724.

41. **Weber-Ban, E. U., B. G. Reid, A. D. Miranker, and A. L. Horwich.** 1999. Global unfolding of a substrate protein by the Hsp100 chaperone ClpA. Nature **401:**90-93.

42. **Wei, B., S. Shin, D. LaPorte, A. J. Wolfe, and T. Romeo.** 2000. Global regulatory mutations in *csrA* and *rpoS* cause severe central carbon stress in *Escherichia coli* in the presence of acetate. J. Bacteriol. **182:**1632–1640.

43. **Wösten, M. M. S. M.** 1998. Eubacterial sigma-factors. FEMS Microbiol Rev **22:** 127-150.

44. **Wurmbach, E., T. Yuen, B. J. Ebersole, and S. C. Sealfon.** 2001. Gonadotropin-releasing hormone receptor-coupled gene network organization. J. Biol. Chem. **276:**47195-47201.

45. **Xu, Y., and Xu, D.** 2000. Protein threading using PROSPECT: Design and evaluation. Proteins. **40:**343-354.

46. **Yura, T., M. Kanemori, and M. T. Morita.** 2000. The heat shock response: regulation and function, p. 3-18. *In* G. Storz, and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, D.C.

47. **Yura, T., and K. Nakahigashi.** 1999. Regulation of the heat-shock response. Curr. Opin. Microbiol. **2:** 159-165.

48. **Zhou, Y.**, **N. Kusakawa, J. W. Erickson, C. A. Gross, and T. Yura.** 1988. Isolation and charaterization of *Escherichia coli* mutants that lack the heat shock sigma factor σ^{32}. J. Bacteriol. **170:**3640-3649.

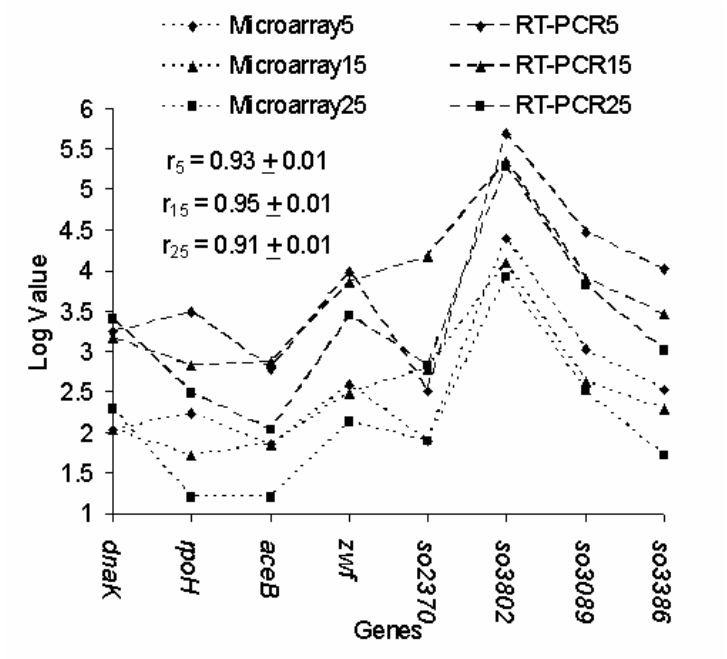

Microarray5
RT-PCR5
Microarray15
RT-PCR15
Microarray25
RT-PCR25
$r_5 = 0.93 \pm 0.01$
$r_{15} = 0.95 \pm 0.01$
$r_{25} = 0.91 \pm 0.01$
Log Value
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
dnaK
rpoH
aceB
zwf
so2370
so3802
so3089
so3386
Genes

FIG. 1.

TABLE 1. Comparison of gene expression measurements by microarray and Real-time RT-PCR assays

Gene	Primers for PCR[a]	5min		15min		25min	
		Microarray	RT-PCR[b]	Microarray	RT-PCR	Microarray	RT-PCR
aceB	gcggagagattgaatacacg/ cttttaaatgcaggccacg	4.1	9.4 ± 0.88	4.1	8.9 ± 0.86	4.9	10.5 ± 0.99
dnaK	cgtgacgtgaacatcatgc/ cagaaacctgtggtggagc	4.7	11.2 ± 1.11	3.3	7.1 ± 0.74	2.3	5.6 ± 0.49
rpoH	taccaccaaagcacaacg/ cctaagttttccgccacc	3.6	6.9 ± 0.56	3.6	7.3 ± 0.59	2.3	4.1 ± 0.43
SO2370	caaggtaatttcagaaagag/ acatgtgacaacggtctacg	6.0	15.7 ± 1.72	5.6	14.4 ± 1.35	4.4	10.9 ± 1.03
SO3089	caaagcccgtacttttgg/ gttgaatactcggccacg	3.7	5.7 + 0.56	6.9	18.1 + 1.99	3.7	7.1 + 0.59
SO3386	gatggtagcttggatgtcacc/ tggggatttagcacacagc	21	51.4 + 7.32	17	40.6 + 2.93	15	38.8 + 3.54
SO3802	atcatcctgaccacccattacc/ ctttcatgctcgtgcattcc	8.1	22.3 + 2.56	6.2	14.8 + 1.33	5.7	14.1 + 2.01
Zwf	gataaccgcaccattgacc/ catatcgcgcatttgacc	5.8	16.2 + 1.98	4.9	11.0 + 1.24	3.3	8.0 + 0.77

[a] Primers used for Real-time RT-PCR are listed as sense/antisense;
[b] Real time RT-PCR values for each gene are presented as the mean Treatment/Control ratio ± standard error;

TABLE 2. Induction of *S. oneidensis* homologs of known heat shock proteins

Gene	Microarray results (fold induction at time indicated) 5min	15min	25min
dnaK	4.7/11.2[a]	3.3/7.1	2.3/5.6
dnaJ	3.8	4.6	3.4
grpE	5.9	5.2	1.8
groES	3.6	3.2	3.8
groEL	2.4	2.3	2.3
clpB	9.0	6.8	4.5
htpG	5.9	4.6	4.3
ftsH	3.3	2.9	1.4
lon	3.8	3.2	2.2
rpoH	3.6/6.9	3.6/7.3	2.3/4.1
rpoE	1.6	1.4	1.2

[a] Microarray value/Real-Time PCR value

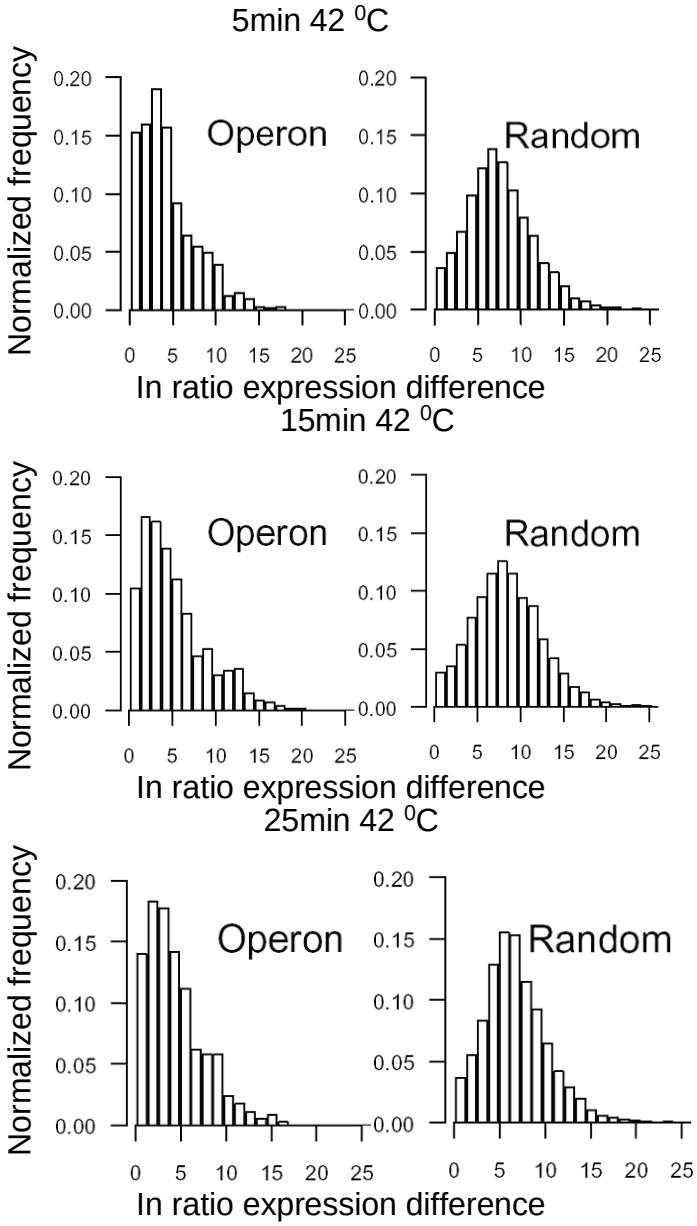

5min 42 0C
Normalized frequency
Operon
Random
0.20
0.15
0.10
0.05
0.00
0 5 10 15 20 25
In ratio expression difference
15min 42 0C
Normalized frequency
Operon
Random
In ratio expression difference
25min 42 0C
Normalized frequency
Operon
Random
In ratio expression difference

FIG. 2.

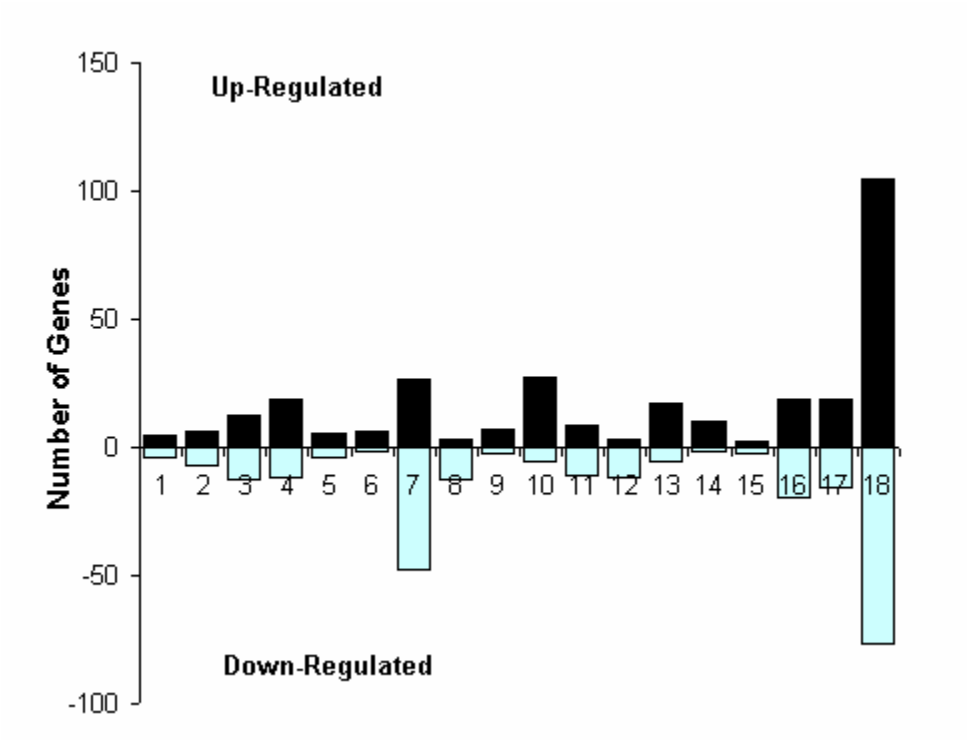

150
100
50
0
-50
-100
Number of Genes
Up-Regulated
Down-Regulated
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

FIG. 3.

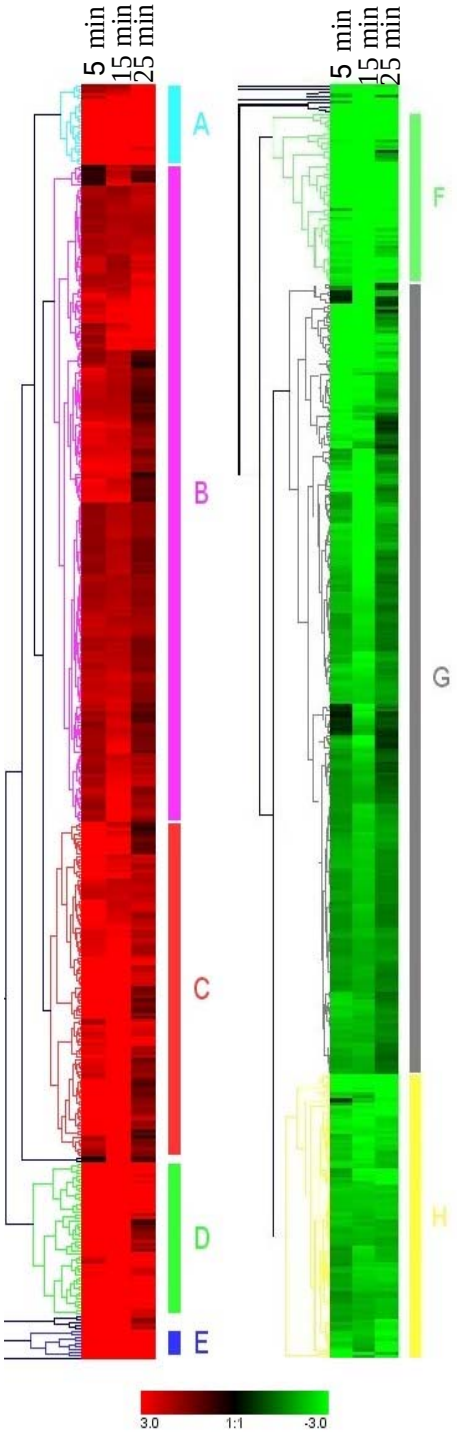
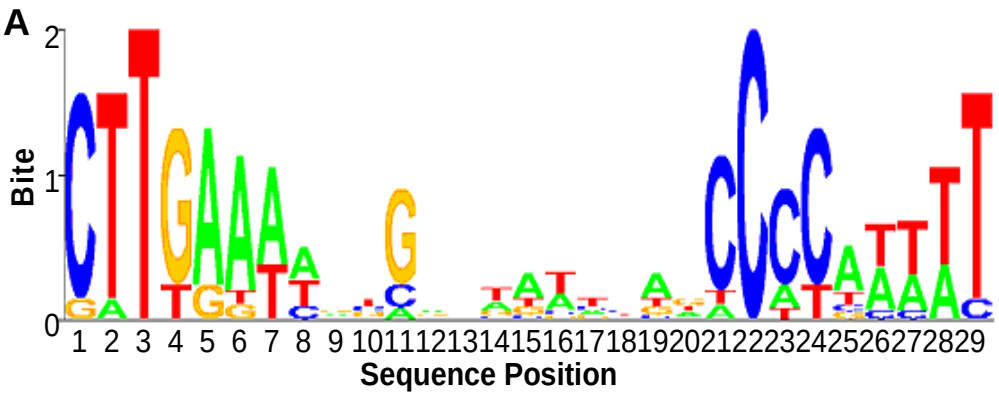


FIG. 4.

A



B

dnaK	ACCGTGGACTTGAAAAAAAATGCGTCCGGCCCCATATCT
groE	TAAAGGCACTTGGATCTGGCGGGGGTGAACCCCATATCA
htpG	TCTTTCCCCTTGAAAAGTGG-ATTTGCAGCCCCATTTTA
grpE	CCTTAGGCCTTGAAACGTCA-AAATTGATCCCCATAATA
clpB	CCATATAGCTTGAATTTGGT-TAAATAGCCCCCATCTTT
clpP	AAGCTAAGCTTGACTTGATT--AGCAGTTCGCCATTTAT
htpX	ATTAGCGAGTAGAAAAACTCTTATCTTTACCCCTTGAAT
ibpA	TTTTTTCCCTTGAAATCCGT-TTTCCTATCCTTATATCT
hslV	TGCAATAACTTGAATTCTGG-CTATCCATCCCCATATTT
rrmJ	GAGTTACTGTTGAAAAACCG-CTATTCTACCCTTATATA
lon	TATTGACCATTGAAAGGGCA--TAAACCGCCCCAATATA
so2017	TCGGGGCACTTGAGTTGAGACGCAAGTGCCCCGATTTAC
	* * * *
consensus	
E. coli	T--T--C-CTTGAAA 13-15 bp C--CCCCAT-T
S. oneidensis	T--T--C-CTTGAAA 13-15 bp CCCCAT-T

FIG. 5.

TABLE 3. Induction of certain hypothetical protein genes[a]

Gene	5 min	15 min	25min	a.a[b]	possible functions[c]
so0367	6.27	4.38	5.92	371	Fic protein family, involved in cell division
so0499	3.08	2.44	2.61	186	
so0503	3.44	5.35	3.91	358	Predicted permease
so0709	3.50	4.07	1.98	305	
so0850	4.96	4.54	1.18	542	
so1094	8.29	8.38	8.75	128	transcription elongation factors
so1264	3.61	5.00	4.82	435	sarcosine oxidase, beta subunit family
so1267	2.50	4.18	3.03	253	GMP synthase
so1274	3.91	5.44	4.75	428	sarcosine oxidase, beta subunit family
so1317	4.44	5.58	3.17	142	
so1419	3.30	2.41	2.01	450	contains a FMN-binding domain
so1432	3.29	3.19	2.02	155	
so1442	5.83	9.68	5.16	489	
so1443	4.15	3.76	4.44	215	
so1454	3.81	2.50	2.31	120	
so1768	5.70	5.19	1.24	240	TraY family, involved in bacterial conjugation
so1787	3.54	4.96	4.13	198	
so2017	3.96	3.00	2.49	287	thioredoxin, Energy metabolism
so2861	3.25	3.27	1.68	283	protein family including the E. coli yfcH
so2862	4.03	3.66	1.65	401	HDIG domain protein
so2863	4.26	5.39	1.87	160	
so3274	4.22	3.92	4.26	159	
so3298	4.52	5.51	3.91	374	
so3335	5.06	4.05	1.03	274	
so3381	3.84	3.90	3.99	416	flavin-containing amine oxidase
so3386	20.82	16.3	15.4	317	Protein of unknown function (DUF523)
so3507	5.18	6.40	3.78	300	BadF/BadG/BcrA/BcrD ATPase family
so3514	3.14	10.1	2.58	874	TonB-dependent out membrane receptor
so3542	5.81	4.53	2.65	788	D-fructose 6-phosphate phosphoketolase
so3682	4.68	3.28	2.55	132	
so3764	4.38	5.03	3.85	137	
so3765	3.88	3.99	3.17	229	PspA/IM30 family
so3766	3.40	3.25	3.11	217	
so3873	2.43	3.83	2.49	251	
so4046	3.39	5.12	2.52	168	
so4161	6.55	5.65	1.59	549	
so4260	9.94	11.2	1.88	239	
so4261	5.03	7.34	2.45	216	
so4356	5.44	6.36	7.64	672	
so4492	6.07	5.41	0.80	170	
so4592	3.25	2.47	1.80	120	

[a] Expression of listed genes encoding hypothetical proteins (>100 a.a) is upregulated more than 2fold in at least two conditions. The cutoff P-value of t-test is 0.05.
[b] Protein length. Information from www.tigr.org.

[c] Predicted functions listed represent ones with the highest match value from www.tigr.org, and blank for no predicted functions.