Original Article

Real-time PCR Analysis of the Heat-Shock Response of *Acidithiobacillus ferrooxidans* ATCC 23270

(*A. ferrooxidans* / heat-shock response / real-time PCR/ transcription regulation)

S. XIAO, J. CHAO, W. WANG, F. FANG, G. QIU, X. LIU

Key Laboratory of Biometallurgy of Ministry of Education, School of Minerals Processing and Bioengineering, Central South University, Changsha, Hunan, China

Abstract. As one of the most important bioleaching bacterial species, *Acidithiobacillus ferrooxidans* is subjected to different kinds of environmental stress such as temperature changes, presence of some toxic heavy metals or pH changes, which normally take place in bioleaching operations and may affect the activity of the bacteria. To obtain further knowledge of the protective mechanisms employed by *A. ferrooxidans* in response to elevated growth temperature, temporal gene expression profiles were examined in cells subjected to heat stress by using real-time PCR. The majority of predicted genes, including those encoding chaperones and heat-shock proteins, were significantly up-regulated ($P < 0.05$ and the fold change ≥ 2.0) over a 25-min period after upshift to the heat-shock temperature (from 30 to 42 °C) and then decrease to readjust their transcript levels to a new steady state at 42 °C. Finally, based on the gene expression data and computational analysis, a putative regulatory site having T-T-T-T-T-T-n-n in the -35 region and n-T-A-T-n-A-T-C in the -10 region with average 19 base pairs separating the two elements was predicted upstream of a number of heat-inducible genes.

Introduction

Acidithiobacillus ferrooxidans (*A. ferrooxidans*, formerly *Thiobacillus ferrooxidans*) is the Gram-negative, acidophilic, chemolithoautotrophic bacterium that obtains its energy from the oxidation of ferrous iron, elemental sulphur, or partially oxidized sulphur compounds (Leduc and Ferroni, 1994; Rohwerder et al., 2003), even hydrogen or formate in anoxic conditions (Drobner et al., 1990; Pronk et al., 1991). The ability of *A. ferrooxidans* to solubilize metal sulphides has been successfully applied in bioleaching operations (Tuovinen, 1990; Rawlings, 2002). However, little is known about the molecular basis underlying the microorganism's perturbation response behaviour or the impact of environmental stresses (e.g., temperature upshift, pH fluctuations, presence of toxic heavy metals and nutrient limitation), which normally take place in bioleaching operations and may affect the bacteria activity (Tuovinen and Kelly, 1972; Brierley, 1978).

Variation in growth temperature is a common stress encountered in nature and industrial application. The heat-shock response, which is elicited by a sudden increase in growth temperature, has been widely used as a model system for studying the impact of stress on biological systems (Mager and De Kruijff, 1995), such as *Escherichia coli*, *Desulfovibrio vulgaris*, *Shewanella oneidensis* and *Campylobacter jejuni* (Arsene et al., 2000; Stintzi, 2003; Gao et al., 2004; Zhang et al., 2006). The hallmark of this adaptive cellular response is that bacteria reduce the gene expression of most normal cellular proteins, inducing at the same time transient overproduction of a limited set of proteins, called heat-shock proteins (Hsps), which are also found to be involved in cellular response to many other stress conditions, such as puromycin and acid stress (Leverrier et al., 2004). Several Hsp families can be designated according to their average apparent molecular mass, e.g., Hsp100, Hsp90, Hsp70 (DnaK), Hsp60 (GroEL), and small Hsps. In addition, ATP-dependent proteases such as ClpP and Lon are known to be Hsps (Gottesman, 1996). In general, their main function in the cell is to mediate the correct assembly of some oligomeric proteins from their subunits (Nilsson and Anderson, 1991). Hsps also play important roles in protein folding, degradation and transport across membranes.

Real-time PCR has become one of the most widely used methods for gene quantitative analysis (Marisa and

Received December 11, 2007. Accepted December 30, 2008.

This research was supported in part by the National Natural Science Foundation of China (No. 50621063, No. 30428014), and the National Basic Research Program (No. 2004CB619201).

Corresponding author: Xueduan Liu, School of Minerals Processing and Bioengineering, Central South University, Changsha, 410083, Hunan, P. R. China. Phone: +86-731-8830546; Fax: +86-731-8710804; e-mail: xueduanliu@yahoo.com.

Abbreviations: ATCC – American Type Culture Collection, Hsps – heat-shock proteins, PCR – polymerase chain reaction, RNAP – RNA polymerase.

Juan, 2005). In this study, expression profiles of a part of predicted genes that encoded molecular chaperones, chaperonin and Hsps were investigated in *A. ferrooxidans* ATCC 23270 in response to a temperature upshift from 30 to 42 °C over a period of 15, 25, and 40 min, and based on the gene expression data and computational analysis, the putative consensus sequence of these heat-inducible genes was predicted.

Material and Methods

Strains, media and culture conditions

A. ferrooxidans ATCC 23270 was obtained from the American Type Culture Collection (ATCC) and grown at 30 °C on a rotary platform (170 rpm) in ATCC 2039 *A. ferrooxidans* medium, which contained 20 g/l $FeSO_4 \cdot 7H_2O$, 2.0 g/l $MgSO_4 \cdot 7H_2O$, 0.80 g/l $(NH_4)_2SO_4$, 0.40 g/l K_2HPO_4 and 5.0 ml/l Wolfe's Mineral Solution. The Wolfe's Mineral Solution was composed of the following components: 1.5 g/l nitrilotriacetic acid; 3.0 g/l $MgSO_4 \cdot 7H_2O$; 0.50 g/l $MnSO_4 \cdot H_2O$; 1.0 g/l NaCl; 0.10 g/l $CaCl_2$; 0.10 g/l $FeSO_4 \cdot 7H_2O$; 0.10 g/l $CoCl_2 \cdot 6H_2O$; 0.10 g/l $ZnSO_4 \cdot 7H_2O$; 0.01 g/l H_3BO_3; 0.01 g/l $CuSO_4 \cdot 5H_2O$; 0.01 g/l $AlK(SO_4)_2 \cdot 12H_2O$ and 0.01 g/l $NaMoO_4 \cdot 2H_2O$. The medium had been adjusted to pH 2.3 before adding $FeSO_4 \cdot 7H_2O$. Growth was followed by direct cell counting and iron oxidation rate (American Public Health Association, 1989).

Heat-shock treatment

After 48 h, when the culture was in log-phase growth, samples (zero time) were taken from the 300-ml culture, a 150-ml aliquot was transferred to a 250-ml flask prewarmed to 42 °C and then incubated in a 42 °C water bath shaker. A parallel identical experiment was performed with a prewarmed 250-ml flask at 30 °C for control. Preliminary experiments were carried out to determine proper heat-shock conditions with samples at 5, 15, 25, 40, and 60 min at 37, 42 or 55 °C. Samples were removed from cultures grown at 30 and 42 °C at 15, 25, and 40 min, filtrated to discard jarosite and centrifuged for 10 min at 12,000 rpm speed in a 5415R centrifuge (Eppendorf AG, Hamburg, Germany). The culture supernatant was removed instantly and the tubes containing the cell pellet were placed in liquid nitrogen for 30 s.

Total RNA extraction and cDNA synthesis

Total RNA was isolated using the TRIzol® reagent (Invitrogen, Carlsbad, CA) and purified with the RNeasy® mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The integral nature of total RNA was checked by 1.5% formaldehyde-agarose gel electrophoresis. Total RNA was quantified at OD_{260} and OD_{280} with NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and then served as the template to synthesize cDNA with SuperScript II RNase H-Reverse Transcriptase (Invitrogen) and Random Primers (Invitrogen). cDNA samples were diluted to a concentration of 200 ng/µl.

Primers design

Real-time PCR primers were designed by using Primer Premier 5.0 and then synthesized by Sagon Biotech (Sagon, Shanghai, China). According to the annotation of *A. ferrooxidans* ATCC 23270 genes from Comprehensive Microbial Resource of The Institute for Genomic Research (TIGR, http://cmr.tigr.org/tigr-scripts/CMRGenomePage), genes that encode molecular chaperones, chaperonin and Hsps relating to protein folding, stabilization and degradation were selected. All primer pairs are listed in Table 1.

The specific fragments were amplified and purified, and then checked by 1.5% agarose gel electrophoresis to detect whether PCRs contained a single product of the expected size. DNA sequencing was carried out by Sagon Biotech, BLAST analysis in TIGR to check the products.

Real-time PCR detection

Each real-time PCR mixture (final volume 50 µl) contained 25 µl of SYBR® Green Real-time PCR Master Mix (Toyobo co., LTD., Osaka, Japan), 2 µl of a 10 mM sense/anti-sense primer, 10 µl of cDNA template, and 11 µl of nuclease-free water. The real-time PCR was carried out with the iCycler iQ Real-time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA): 1 cycle of 95 °C for 30 s, and then 40 cycles of 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s. At the completion of each run, melting curves for the amplicons were measured by raising the temperature by 0.5 °C from 55 to 95 °C while monitoring fluorescence. The specificity of the PCR amplification was checked by examining the derivatized melting curve for Tm, its symmetry and the lack of non-specific peaks. Three parallel measurements for each cDNA sample from independent RNA isolation were detected. The gene expression ratio was recorded as the fold difference in quantity from samples that grew at the treatment (42 °C) versus control temperature (30 °C). The results were normalized against house-keeping gene 16S rRNA to correct the sample-to-sample variation.

Computational promoter analysis and motif identification

To search for potential regulatory motifs upstream of heat-inducible genes, the Neural Network Promoter Prediction software from the Berkeley Drosophila Genome Project (http://www.fruitfly.org/seq_tools/promoter.html) was used to predicted promoter site (Schneider and Stephens, 1990; Crooks et al., 2004). Based on the distance between the genes, the similarity of the cluster of orthologous groups of protein functional classes assigned to each gene, the similarity of codon usage and other factors, regions representing approximately 500 base pairs of the DNA sequences upstream of the translational start site of genes responsive to heat-shock stress were extracted from the TIGR genome database.

Table 1. The primers for real-time PCR detection

Locus	Gene symbol	Primers (Sense primer/Anti-sense primer: 5'-3')	Tm (°C)	Size of products (bp)
AFE_0356	*rpoH*	GCAACGCAAACTGTTCTTCA GCAACGCAAACTGTTCTTCA	55.75 59.42	184
AFE_0358	*hslU*	CGCCATCCTGGGTAAAGTG TGGAGTTGGAGGCCCTGAG	59.72 61.88	129
AFE_0440	*dnaK*	ATCACCGTACCCGCCTACT TGTTCACCCTCCATTTCAGC	59.72 57.80	224
AFE_0441	*dnaJ*	GAGGCGGCTCTGGGTAAA AGGGACGGGTAACGGAAA	59.58 57.30	169
AFE_1009	AFE_1009	ACAGCGGGTGATTGGTC TTCTCCTGCTTCCGTTCC	57.01 57.30	152
AFE_1437	AFE_1437	GCGCATATTGATGTCCTTGAT TCTTCCTTGACGCCACTGAT	56.06 57.80	128
AFE_1673	*hslO*	TCGCCTGGATGATATTTACG TCAACGGTCCCTTGCTCT	55.75 57.30	161
AFE_2172	AFE_2172	TCCGCAGAACTGCAAACG CGGCGAAAGGACAAATGG	57.30 57.30	140
AFE_2327	*hypC-1*	TACGGCGGGAGGTCAATA AGCACATCCAGCGTCAGG	57.30 59.58	142
AFE_2363	*hscA*	CGTACAGTGGCGGGTGAAA GGGCCTCGTCGAAATAAGC	59.72 59.72	139
AFE_2495	*groES*	TCATCATTCCCGACACCG TCACCAGCAGTTCTTCACCT	57.30 57.80	180
AFE_2496	*groEL*	CCCACCATTACCAAGGACG CGACGACGATGACCGATTT	59.72 57.56	244
AFE_2781	*htpX-2*	ACTGGAATGATGAGCCTAACG GGCGAGCATGGACAACC	58.01 59.42	215
AFE_3174	*htpG*	GAATGGGAAACGGTCAATAA CAGGAATGAAGAGTAAGGAGGT	53.70 58.21	178
AFE_0254	*16S*	AATCCAAGAAGAAGCACCG CCACTGATGTTCCTCCAG	55.41 57.30	238

The multilevel consensus sequences for each promoter motif were used to generate a sequence logo by AlignACE (Roth et al., 1998; Hughes et al., 2000. http://weblogo.Berkele.edu/logo.cgi) that was a graphical representation of nucleic acid multiple sequence alignment. Each logo consists of stacks of symbols, one stack for each position in the sequence. The overall height of the stack indicates the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino or nucleic acid at that position (Waibel et al., 1989).

Results

Response of A. ferrooxidans 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. Table 2 shows the temporal expression levels for some of the *A. ferrooxidans* Hsps; the temporal gene expression analysis indicated that the general changes in mRNA levels upon the temperature increase were largely transient. *A. ferrooxidans* cells responded with large changes in the expression level of selected genes (≥ 2-fold) during the first 25 min. The data from the preliminary experiments showed that almost all of these genes remained differentially expressed at 60 min (data not shown). These gene expression profiles suggest that *A. ferrooxidans* quickly readjusts its transcript levels to a new steady state at the heat-shock temperature (42 °C), thereby allowing the bacterium to survive in the stress. This is in agreement with previous findings reported for *E. coli*, *C. jejuni* and *S. oneidensis* (Richmond et al., 1999; Stintzi, 2003; Zhang et al., 2006).

Computational prediction of consensus motif in A. ferrooxidans

To predict genes regulated by the σ32 transcription factor, DNA sequences upstream of heat-inducible genes were analysed for potential regulatory motifs. Sequence analysis indicated that a few genes are likely encoded

Table 2. List of genes with differential expression induced by heat shock (P < 0.05)

Locus	Gene symbol	Annotated function (TIGR)	Fold change 15 min	25 min	40 min
AFE_0356	*rpoH*	RNA polymerase σ32 factor	7.73 ± 1.18	16.06 ± 1.58	2.69 ± 0.40
AFE_0358	*hslU*	heat-shock protein HslVU, ATPase subunit HslU	3.31 ± 0.55	23.90 ± 2.26	11.75 ± 1.18
AFE_0440	*dnaK*	chaperone protein Dnak (dnaK)	11.86 ± 1.26	13.79 ± 1.18	5.97 ± 0.89
AFE_0441	*dnaJ*	chaperone protein DnaJ (dnaJ)	8.66 ± 0.99	19.99 ± 1.38	14.87 ± 1.45
AFE_1009	AFE_1009	heat-shock protein, Hsp20 family	0.96 ± 0.20	0.76 ± 0.17	1.12 ± 0.15
AFE_1437	AFE_1437	heat-shock protein, Hsp20 family	13.65 ± 1.24	25.64 ± 2.92	24.11 ± 1.73
AFE_1673	*hslO*	33 kDa chaperonin (hslO)	0.87 ± 0.23	2.55 ± 0.33	2.01 ± 0.31
AFE_2172	AFE_2172	heat-shock protein, Hsp20 family	4.47 ± 0.60	4.51 ± 0.82	11.30 ± 1.50
AFE_2327	*hypC-1*	hydrogenase assembly chaperone HypC (hypC-1)	3.84 ± 0.66	12.97 ± 1.76	3.84 ± 0.25
AFE_2363	*hscA*	Fe-S protein assembly chaperone HscA (hscA)	2.33 ± 0.52	14.32 ± 1.11	8.29 ± 0.87
AFE_2495	*groES*	chaperonin, 10 kDa (groES)	8.22 ± 1.13	12.81 ± 1.51	14.24 ± 1.64
AFE_2496	*groEL*	chaperonin, 60 kDa (groEL)	10.97 ± 1.09	22.89 ± 2.01	12.10 ± 2.52
AFE_2781	*htpX-2*	heat-shock protein HtpX (htpX-2) [3.4.24.-]	2.10 ± 0.57	3.81 ± 0.42	5.45 ± 0.84
AFE_3174	*htpG*	heat-shock protein HtpG (htpG)	7.86 ± 1.08	8.75 ± 0.92	12.55 ± 1.92

within operons, including *dnaK*/*dnaJ* and *groES*/*groEL*, while others are divergently transcribed. A subset of 10 intergenic sequences (500-base each in length or the non-coding sequence between two genes) containing putative promoter regions were searched using the Neural Network Promoter Prediction software for putative conserved DNA motifs associated with heat-inducible genes.

This analysis showed that the σ32-controlled promoters have T-T-T-T-T-T-n-n in the -35 region and n-T-A-T-n-A-T-C in the -10 region with average 19 bp separating the two elements (Fig. 1). Table 3 revealed that in 8 out of the 10 genes containing this motif, it was located within 100-base upstream of the putative start codon, which is within the preferred region for binding of regulatory proteins (Thompson et al., 2003). Compared with the *E. coli* σ32 binding site, the motif had a weak similarity to 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 in *E. coli*, and was unlike the motifs of *S. oneidensis* (Gao et al., 2004) or *D. vulgaris* (Zhang et al., 2006) either; these observations suggest that maybe only stronger or weaker interactions of the sigma factors with their respective binding sites might take place. Some experimental test of this prediction is in progress, and then we will blast against the *A. ferrooxidans* genome to identify new potential heat-shock proteins under a similar control mechanism.

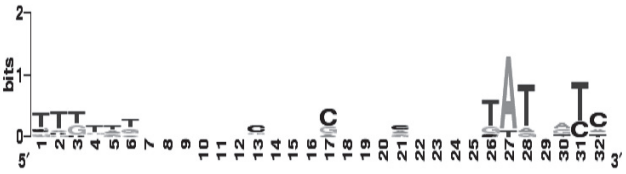

Fig. 1. The motif model of putative promoter, which has T-T-T-T-T-T-n-n in the -35 region and n-T-A-T-n-A-T-C in the -10 region with average 19 base pairs separating the two elements, was predicted upstream of a number of putative heat-inducible genes of *A. ferrooxidans*; the motif is represented by a sequence logo generated by the Web Logos software (http://weblogo.Berkeley.edu/logo.cgi).

Discussion

Although 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

Table 3. Motif sequence and promoter score

Locus	Gene symbol	Motif sequence (5'-3')	Score[a]	Distance[b] (bp)
AFE_0358	*hslU*	5'AGCAGTGTTTCAAATACTACAGTACAAGTTATAGCACCGC3'	0.89	35
AFE_0440/0441	*dnaK/dnaJ*	5'GGTCTTGAAATACCCTGGTCCTTTCCCCATATGGTCTTGG3'	0.87	27
AFE_1437	AFE_1437	5'ATTTTTTCGGGAGTGCGGTTGAACACCTTGATATTCTCTC3'	0.88	254
AFE_1673	*hslO*	5'CCTTTTTGTGATTGCATCCGCCGGGGTGGTAGTACCCGCC3'	0.96	25
AFE_2172	AFE_2172	5'GCCTGGTTGTCGCCGCCAGGCTGGAGAGGTATAGCCGCAA3'	0.95	52
AFE_2327	*hypC-1*	5'CATGTTTTATATATCTAGTTGTTCAATAACAATATTATTG3'	0.93	67
AFE_2363	*hscA*	5'ATTTCATTGACCCGGTCGAAGCTGCGGTTGATGATATCGC3'	0.95	130
AFE_2495/2496	*groES/groEL*	5'CCGCCCCTTGACAGGGCATTCTCAGCGCCTATGATTAGCA3'	0.92	73
AFE_2781	*htpX-2*	5'TGGGTTGTTTAGCCATCTGGCAGGGCGAGTATCGTCACCG3'	0.85	62
AFE_3174	*htpG*	5'TGCTTTGGATGAAGCACCCGCGGCCTTGATTTTTTCTTCG3'	0.85	77

[a] the promoter score, which is closer to 1.0, the result is more credible
[b] distance from the start codon (bp) of the genes

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 (Richmond et al., 1999).

The genes which have predicted functions related to protein fate include mainly those encoding chaperones, chaperonins and Hsps, several of them have been well characterized in other bacteria and encode DnaK, DnaJ, GroEL, GroES, GrpE, HtpG, and Lon/La proteases. Also present in the *A. ferrooxidans* ATCC 23270 genome are genes predicted to encode known regulators of the heat-shock response, namely, σ32 and σE. In *E. 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 (σ32), directly responsible for regulation of the heat-shock response (Straus et al., 1987) and encoded by *rpoH*, which complexes with the core RNA polymerase (RNAP) and directs the RNAP holoenzymes to transcribe specifically from heat-regulated promoters (Straus et al., 1987; Yura et al., 2000), thus permitting both steady-state and stress-induced levels of Hsp expression (Zhou et al., 1988). The increase in the intracellular concentration of the σ32 transcription factor is due to a concomitant increase in both the stability and synthesis of σ32.

In our study, σ32 had a rapid induction at the early stage and kept the high level for the first 25 min after the temperature upshift and then decreased; that was maybe because of increasing *hslU* expression, which in *E. coli* encoded heat-shock proteins HslU, as the subunit of HslVU protease degrading σ32.

The major heat-shock proteins from *A. ferrooxidans* had been identified as DnaK and GroEL, encoded by *dnaK* and *groEL*, which showed 80% and 70% identity to DnaK and GroEL from *E. coli* (Varela and Jerez, 1992). The DnaK-DnaJ-GrpE chaperone system (hsp70 heat-shock system) and GroEL/GroES chaperone system joined in protein folding. The expression of chaperone system genes increased immediately after the temperature upshift and then decreased.

Interestingly, AFE_1009, AFE_1437, AFE_2172 with the same putative function belong to Hsp20 family genes, and AFE_1009 expression stayed at the former level (0.5 to 2.0-fold), which infers that AFE_1009 possibly is not under the control of σ32 in response to the heat shock from 30 to 42 °C.

Other genes encoding chaperonin, chaperone and Hsps involved in protein folding, stabilization and degradation were induced by the heat shock immediately. *hypC-1* encodes hydrogenase assembly chaperone HypC, binding nickel ion with metallochaperone activity, as components of nickel-dependent hydrogenase involved in electron transport. *hscA*, the Fe-S protein assembly chaperone gene, is a member of the iron-sulphur cluster assembly *iscSUA-hscBA-fdx* system.

Our future work will focus on global transcriptome analysis of the heat-shock response of *A. ferrooxidans* to obtain further knowledge of the protective mechanisms and to find out how to enhance the resistance to heat shock and other environmental stress by gene engineering.

Acknowledgements

A. ferrooxidans ATCC 23270 preliminary sequence data and ATCC medium 2039 composition were obtained from The Institute for Genomic Research website at http://www.tigr.org, and American Type Culture Collection website at http://www.atcc.org, respectively.

References

American Public Health Association (APHA). (1989) *Standard Methods for the Examination of Water and Wastewater*, 17th ed., J. Am. Public Health Assoc., Washington, D.C.

Arsene, F., Tomoyasu, T., Bukau, B. (2000) The heat shock response of Escherichia coli. *Int. J. Food Microbiol*. **55**, 3-9.

Brierley, C. L. (1978) Bacterial leaching. *CRC Crit. Rev. Microbiol.* **6**, 207-26I.

Crooks, G. E., Hon, G., Chandonia, J. M., Brenner, S. E. (2004) WebLogo: A sequence logo generator. *Genome Res.* **14**, 1188-1190.

Drobner, E., Huber, H., Stetter, K. O. (1990) Thiobacillus ferrooxidans, a facultative hydrogen oxidizer. *Appl. Environ. Microbiol*. **56**, 2922-2923.

Gao, H., Wang, Y., Liu, X., Yan, T., Wu, L., Alm, E., Arkin, A., Thompson, D. K., Zhou, J. (2004) Global transcriptome analysis of the heat shock response of Shewanella oneidensis. *J. Bacteriol.* **186**, 7796-7803.

Gottesman, S. (1996) Proteases and their targets in Escherichia coli. *Annu. Rev. Genet.* **30**, 465-506.

Hughes, J. D., Estep, P. W., Tavazoie, S., Church, G. M. (2000) Computational identification of cis-regulatory elements associated with groups of functionally related genes in Saccharomyces cerevisiae. *J. Mol. Biol.* **296**, 1205-1214.

Leduc, L. G., Ferroni, G. D. (1994) The chemolithotrophic bacterium Thiobacillus ferrooxidans. *FEMS Microbiol. Lett.* **108**, 103-120.

Leverrier, P., Vissers, J. P., Rouault, A., Boyaval, P., Jan, G. (2004) Mass spectrometry proteomic analysis of stress adaptation reveals both common and distinct response pathways in Propionibacterium freudenreichii. *Arch. Microbiol.* **181**, 215-230.

Mager, W. H., De Kruijff, A. J. J. (1995) Stress-induced transcriptional activation. *Microbiol. Rev.* **59**, 506-531.

Marisa, L. W., Juan, F. M. (2005) Real-time PCR for mRNA quantitation. *BioTechniques* **39**, 75-85.

Nilsson, B., Anderson, S. (1991) Proper and improper folding of proteins in the cellular environment. *Annu. Rev. Microbiol.* **45**, 607-635.

Pronk, J. T., Meijer, W. M., Hazeu, W., van Dijken, J. P., Bos, P., Kuenen, J. G. (1991) Growth of Thiobacillus ferrooxidans on formic acid. *Appl. Environ. Microbiol.* **5**, 2057-2062.

Rawlings, D. E. (2002) Heavy metal mining using microbes. *Annu. Rev. Microbiol.* **56**, 65-91.

Richmond, C. S., Glasner, J. D., Mau, R., Jin, H., Blattner. F. R. (1999) Genome-wide expression profiling in Escherichia coli K-12. *Nucleic Acids Res.* **27**, 3821-3835.

Rohwerder, T., Gehrke, T., Kinzler, K., Sand, W. (2003) Bioleaching review part A: progress in bioleaching: fundamentals and mechanism of bacterial metal sulfide oxidation. *Appl. Microbiol. Biotechnol.* **63**, 239-248.

Roth, F. P., Hughes, J. D., Estep, P. W., Church, G. M. (1998) Finding DNA regulatory motifs within unaligned noncoding sequences clustered by whole-genome mRNA quantitation. *Nat. Biotechnol.* **16**, 939-945.

Schneider, T. D., Stephens, R. M. (1990) Sequence logos: A new way to display consensus sequences. *Nucleic Acids Res.* **18**, 6097-6100.

Stintzi, A. (2003) Gene expression profile of Campylobacter jejuni in response to growth temperature variation. *J. Bacteriol.* **185**, 2009-2016.

Straus, D. B., Walter, W. A., Gross, C. A. (1987) The heat shock response of Escherichia coli is regulated by changes in the concentration of σ32. *Nature* **329**, 348-351.

Thompson, W., Rouchka, E. C., Lawrence, C. E. (2003) Gibbs recursive sampler: finding transcription factor binding sites. *Nucleic Acids Res.* **31**, 3580-3585.

Tuovinen, O. (1990) Biological fundamentals of mineral leaching processes. In: *Microbial Mineral Recovery*, eds. Ehrlich, H. L., Brierley, C. L., pp. 55-77, McGraw-Hill Book Co., New York, USA.

Tuovinen, O. H., Kelly, D. P. (1972) Biology of Thiobacillus ferrooxidans in relation to the microbiological leaching of sulphide ores. *Z. Allg. Mikrobiol.* **12**, 311-346.

Varela, P., Jerez, C. A. (1992) Identification and characterization of GroEL and DnaK homologues in Thiobacillus ferrooxidans. *FEMS Microbiol. Lett.* **77**, 149-153.

Waibel, A. H., Hanazawa, T., Hinton, G. E., Shikano, K., Lang, K. J. (1989) Phoneme recognition using time-delay Neural Networks. *IEEE Trans. Acoust. Speech Signal Process* **37**, 328-339.

Yura, T., Kanemori, M., Morita, M. T. (2000) The heat shock response: regulation and function. In: *Bacterial Stress Responses*, eds. Storz, G., Aronis, R. H., pp. 3-18, ASM Press, Washington, D.C..

Zhang W. W., Culley, D. E., Hogan, M., Vitiritti, L., Brockman, F. J. (2006) Oxidative stress and heat-shock response in Desulfovibrio vulgaris by genome-wide transcriptomic analysis. *Anton. Leeuw.* **90**, 41-55.

Zhou, Y., Kusukawa, N., Erickson, J. W., Gross, C. A., Yura, T. (1988) Isolation and characterization of Escherichia coli mutants that lack the heat shock sigma factor σ32. *J. Bacteriol.* **170**, 3640-3649.