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Transcriptional analysis of dynamic heat-shock response by the hyperthermophilic bacterium *Thermotoga maritima*

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Abstract The thermal stress response of the hyperthermophilic bacterium *Thermotoga maritima* was characterized using a 407-open reading frame-targeted cDNA microarray. Transient gene expression was followed for 90 min, following a shift from 80°C to 90°C. While some aspects of mesophilic heat-shock response were conserved in *T. maritima*, genome content suggested differentiating features that were borne out by transcriptional analysis. Early induction of predicted heat-shock operons *hrcA-grpE-dnaJ* (TM0851-TM0850-TM0849), *groES-groEL* (TM0505-TM0506), and *dnaK-sHSP* (TM0373-TM0374) was consistent with conserved CIRCE elements upstream of *hrcA* and *groES*. Induction of the *T. maritima rpoE/sigW* and *rpoD/sigA* homologs suggests a mechanism for global heat-shock response in the absence of an identifiable ortholog to a major heat-shock sigma factor. In contrast to heat-shock response in *Escherichia coli*, the majority of genes encoding ATP-dependent proteases were downregulated, including *clpP* (TM0695), *clpQ* (TM0521), *clpY* (TM0522), *lonA* (TM1633), and *lonB* (TM1869). Notably, *T. maritima* showed indications of a late heat-shock response with the induction of a *marR* homolog (TM0816), several other putative transcriptional regulators (TM1023, TM1069), and two α-glucosidases (TM0434 and TM1068). Taken together, the results reported here indicate that, while *T. maritima* shares core elements of the bacterial heat-shock response with mesophiles, the thermal stress regulatory strategies of this organism differ significantly. However, it remains to be elucidated whether these differences are related to thermophilicity or phylogenetic placement.

Keywords cDNA microarray · *groESL* · Heat shock · *hrcA* · Hyperthermophile · *Thermotoga*

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Introduction

Comparative analysis of sequenced bacterial genomes has revealed substantial conservation of a core set of heat-shock proteins (Koonin et al. 2000). However, the few comprehensive differential gene expression studies reported to date suggest that strategies underlying regulation of heat-shock genes differ somewhat among prokaryotic organisms (Aldsworth et al. 1999; Helmann et al. 2001; Lopez-Garcia and Forterre 2000; Shockley et al. 2003; Stintzi 2003; Yura et al. 2000). The reason for these various regulation strategies is not clear but may be related to the extent of thermal variation normally encountered in a specific organism's natural habitat (Stintzi 2003).

Unlike enteric or soil microorganisms that occupy relatively stable thermal niches, hyperthermophiles, which grow optimally at 80°C or higher, can experience considerable thermal variation in their natural habitats (Stetter 1985). However, the genome sequence of a hyperthermophilic bacterium,*Thermotoga maritima* MSB8, reveals the presence of genes encoding heat-shock proteins that are similar to those found in less thermophilic bacteria. A few *T. maritima* heat-shock proteins have been characterized, including HtrA (TM0571), a molecular chaperone which acts as a protease at elevated temperatures (Kim et al. 2003), and ClpQY/HslUV (TM0521, TM0522), a complex homologous to the archaeal proteasome (Song et al. 2003). The

apparent lack of orthologs to known mesophilic stress-response regulators, such as σ^H or σ^B (Grossman et al. 1984), CtsR (Kruger and Hecker 1998), and HspR (Grandvalet et al. 1997), suggests that *T. maritima* has alternative mechanisms for thermal stress response regulation, perhaps related to the characteristics of its natural environment. To explore this issue, a targeted cDNA microarray was used to follow the dynamic response of *T. maritima* following a temperature shift from 80°C to 90°C. Of particular interest was the differential response of genes corresponding to known and putative heat-shock proteins, putative regulators, and sigma factors.

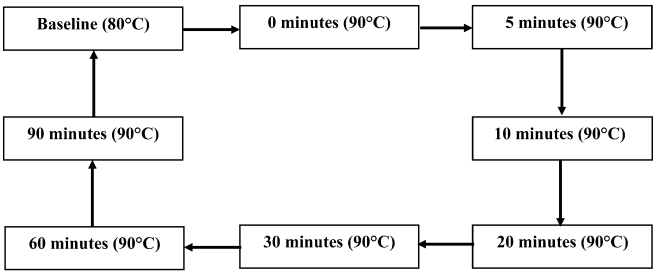


Fig. 1 Experimental strategy showing loop design for *Thermotoga maritima* dynamic heat shock from 80°C to 90°C. *Arrow tails and heads* correspond to aminoallyl labels Cy3 and Cy5, respectively (Cy3 → Cy5)

Materials and methods

Microorganism and growth conditions

Thermotoga maritima (DSM 3109) was cultured anaerobically at 80°C on sea salts medium (SSM), as described previously (Pysz et al. 2001; Rinker and Kelly 2000). Maltose (Sigma, St. Louis, Mo.) was added to SSM (final concentration 5 g/l) as a carbon source prior to inoculation. A 50-ml batch culture was used to inoculate 500 ml of SSM medium supplemented with 5 g/l maltose in a 1-l Pyrex bottle. A 14.0-l fermentor (New Brunswick Scientific, Edison, N.J.) containing 13 l of SSM medium (1.08× concentrated for a final concentration of 1× SSM) and 1 l of 70 g/l maltose (final concentration of 5.0 g/l) was inoculated with 250 ml of the 500-ml batch culture. The fermentor contained an internal temperature controller, and the pH was maintained by a Chemcadet controller (Cole Parmer, Vernon Hills, Ill.). High-purity N_2 was used to reduce the medium and to sparge during inoculation. Following 8 h of growth at 80°C, a sample was collected. The temperature set point was then shifted, taking approximately 2 min to reach 90°C. Samples were taken at 0, 5, 10, 20, 30, 60, and 90 min after reaching 90°C. Approximately 20 ml of culture was collected prior to sampling at each time point to eliminate pre-existing fluid in the sampling lines. At each time point, 500 ml of culture was withdrawn and immediately put on ice until processed for RNA extraction (see below). One milliliter of sample was removed for cell density enumeration by epifluorescent microscopy with acridine orange stain (Hobbie et al. 1977).

RNA extractions and hybridizations

RNA was extracted, as described previously (Chhabra et al. 2003). Concentrations and degree of purity were determined by absorbance at 260 nm and 280 nm, as well as gel electrophoresis (1% agarose gel, 60 V). Procedures for reverse-transcription reactions, aminoallyl labeling with Cy3 and Cy5, and hybridization reactions have been reported elsewhere (Chhabra et al. 2003).

Targeted cDNA microarray analysis

A targeted cDNA microarray representing 407 open reading frames was printed, following protocols described previously (Chhabra et al. 2003). PCR products were randomized within plates before printing using a random number generator, and each PCR product was spotted six times on each array. For purposes of cDNA labeling and data analysis, a loop design (Fig. 1) was utilized to incorporate reciprocal labeling of all time-point samples with both Cy3 and Cy5. Mixed model ANOVA analysis was used to evaluate differential expression data using approaches presented elsewhere (Chhabra et al. 2003). Briefly, least-squares estimates of gene-specific treatment effects, corrected for global and gene-specific sources of error, were used to construct pair-wise differences analogous to fold changes for each gene between all pairs of conditions. The statistical significance of these fold changes was then determined by *t*-test. A Bonferroni correction was used to establish an experiment-wide false-positive rate of $\alpha = 0.05$ by dividing α by 11,396, the number of comparisons performed for all genes over all possible treatment pairs. The corrected false positive rate was 4.39×10^{-6} (corresponding to a $-\log_{10}$ *P*-value > 5.4). Least-squares estimates of gene-specific treatment effects for all time points were used to perform hierarchical clustering using Ward's minimum variance method in JMP 5.0 (SAS Institute, Cary, N.C.).

Regulatory sequence analysis

All promoter searches were performed using the search tools at the Regulatory Sequence Analysis Tools Web site (http://rsat.ulb.ac.be/rsat/) (van Helden et al. 2000). The palindromic CIRCE consensus TTAGCACTC-N9-GAGTGCTAA was used to search the full genome sequence of *T. maritima* for all sequences with two or fewer mismatches to this sequence.

Results and discussion

Dynamic heat-shock response

Figure 2 shows the decline of *Thermotoga maritima* cell density from 3.4×10^8 cells/ml at 80°C to 1.6×10^8 cells/ml at 90°C during the 90 min following heat shock. The maximal growth temperature for *T. maritima* was previously determined to be 90°C (Huber et al. 1986); therefore, this temperature was chosen for heat-shock studies. Due to the difficulty of partitioning the effects of temperature and cell density, the term "heat shock" will be used to refer to the collective effects of the shift in growth temperature and the change in cell density observed here. Dynamic changes in gene expression during heat shock were followed using a targeted cDNA microarray consisting of 407 genes (about 20% of the genome), chosen for their relevance to stress response and related physiological phenomena. Transcriptional response was analyzed at seven time points over the period of 0 min to 90 min following the shift from 80°C to 90°C. Clusters of interest drawn from gene-specific treatment effects for all genes over all time points are shown in Fig. 3. Pair-wise comparisons, which represent fold changes in gene expression compared to the 80°C

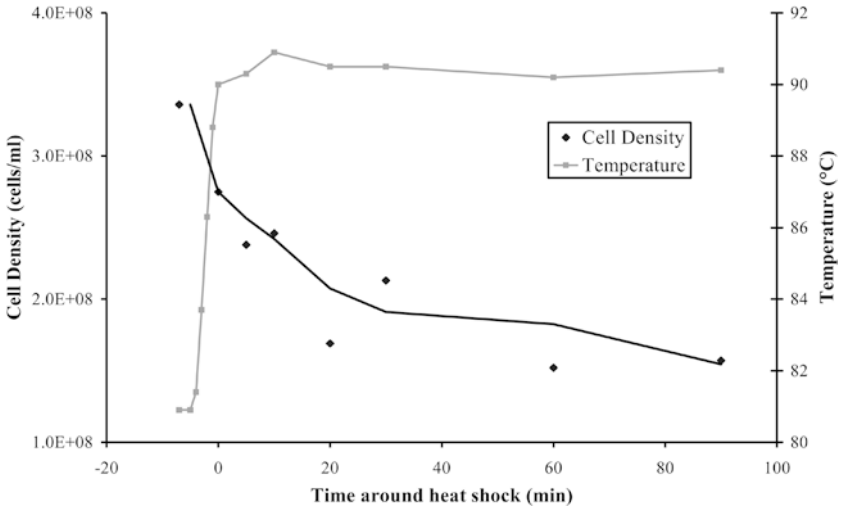


Fig. 2 Response of mid-exponential growth phase *T. maritima* cells to shift from 80°C to 90°C

reference point, are shown for specific genes in Tables 1 (proteases), 2 (sigma factor-associated genes), and 3 (other genes responding to heat shock), which will be available upon acceptance of this manuscript at http://webbie.che.ncsu.edu/extremophiles/microarray/index.html.

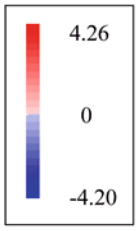
Expression changes of core heat-shock genes

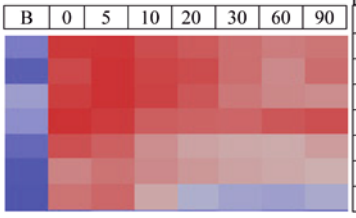
Many of the conserved, core heat-shock genes followed similar expression profiles during thermal stress (see Fig. 3). At 80°C, most suspected heat-shock genes were expressed at low levels but were significantly induced after the 10°C temperature increase (Fig. 3, cluster C1). This phenomenon was observed for genes encoding components of the DnaK chaperone machinery (TM0850, TM0849, TM0373), heat-shock protein class I (TM0374), ATP-dependent protease ClpC-2 frameshift (TM0873), GroESL (TM0505 and TM0506), and heat-shock operon repressor HrcA (TM0851) (Fig. 3). Although *groEL* (TM0506), *clpC-1* ATP-binding subunit (TM0198), and a *malK*-family ATP binding protein (TM1276) displayed higher expression levels at the 80°C baseline than other core heat-shock genes, they were also induced upon heat shock (Fig. 3, cluster C2). The existence of an internal promoter specific to *groEL* (TM0506) cannot be ruled out (data not shown).

Several *T. maritima*genes strongly induced upon heat shock are homologous to *Bacillus subtilis* class I heat-shock genes *hrcA-grpE-dnaJ-dnaK* and *groEL-groES*, regulated by binding of the HrcA repressor to the CIRCE operator (Schulz and Schumann 1996). While sequences with high identity to the CIRCE element are also conserved upstream of the *T. maritima hrcA-grpE-dnaJ* and *groEL-groES* operons, no additional sequences with two or fewer mismatches to the CIRCE consensus could be detected in the genome (see Materials and methods). Transcription of the *B. subtilis hrcA* locus involves multiple transcripts with differential stabilities to ensure appropriate synthesis rates for associated proteins (Homuth et al. 1997). Although stability-mediated transcript regulation from the *T. maritima hrcA* operon has not been examined, the organization of *T. maritima dnaK* with a class I heat-shock protein (TM0374) and no apparent CIRCE element might effect differential regulation of *dnaK* and *dnaJ*. This suggests the operation of an alternate mode of transcriptional activation of these heat-shock genes. The *dnaK* gene has also been observed to be co-transcribed with two small heat-shock protein homologs in *Porphyromonas gingivalis* (Yoshida et al. 1999).

Response of proteases and SOS genes to thermal stress

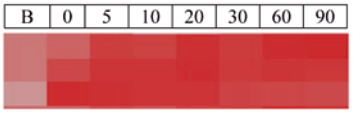
In light of the well-documented induction of proteases in mesophilic bacteria under thermal stress in other microarray experiments (Helmann et al. 2001; Richmond et al. 1999), the lack of differential expression of *T. maritima* proteases under heat shock is striking (Table 1). This may be related to differences in induction mechanisms. In *Escherichia coli*, genes encoding ATP-dependent proteases Lon, ClpA, ClpQ, ClpP, ClpX, ClpY, ClpB, and membrane-bound FtsH are upregulated upon heat shock under control of σ^H-recognized promoters (Yura et al. 2000). The CtsR repressor regulates the class III heat-shock genes of *B. subtilis*, which include the *ClpC* operon, *ClpP*, and *ClpE* (Derre et al. 1999). Orthologous regulators are not apparent in the *T. maritima* genome, which may underlie the diminished response of ATP-dependent proteases to heat shock. The lack of induction of the *T. maritima clpQY*genes (TM0521, TM0522) could be related to the biochemical properties of the corresponding complex, since maximal ATP hydrolysis and peptide breakdown activities have been noted between 75°C and 80°C and were much lower at 90°C (Song et al. 2003). Only the genes encoding ATPase ClpC-1 (TM0198), ATPase ClpC-2 (TM0873, frameshift), FtsH (TM0580), heat-shock serine protease HtrA (TM0571), carboxy-terminal protease





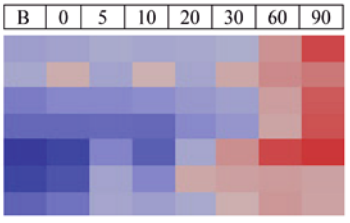
Cluster C1

Locus	Function	Max Fold-Change	Time
TM0373	DnaK	24.9	0
TM0873	ATPase ClpC-2 frameshift	42.6	5
TM0849	DnaJ	14.7	5
TM0374	Heat shock protein class I	20.5	0
TM0505	GroES	22.3	0
TM0851	Heat shock operon repressor HrcA	14.8	5
TM0850	GrpE	19.3	5



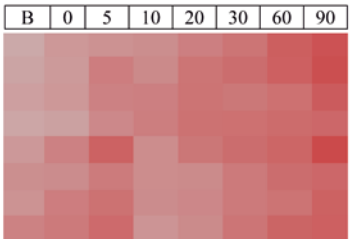
Cluster C2

Locus	Function	Max Fold-Change	Time
TM0198	ATPase ClpC-1	3.8	90
TM1276	ATP-binding ABC transporter (MalK)	3.6	20
TM0506	GroEL	3.9	90



Cluster C3

Locus	Function	Max Fold-Change	Time
TM0434	α-glucosidase	10.0	90
TM0616	AAA+ superfamily ATPase	3.5	90
TM1068	α-glucosidase, Amy4B	13.0	90
TM1069	Transcriptional regulator, GlpR/DeoR family	20.7	90
TM0816	Transcriptional regulator, MarR family	108.0	90
TM0032	Transcriptional regulator, XylR related	10.0	60
TM0300	Transcriptional regulator, LacI family	6.2	60



Cluster C4

Locus	Function	Max Fold-Change	Time
TM0445	Predicted GTPase (COG1160)	4.8	90
TM1761	UvrB, Excinuclease ABC, subunit B	3.3	90
TM0508	Conserved hypothetical (COG2110, COG2256)	4.6	90
TM1719	MutS-like ATPase	3.1	90
TM1451	Sigma A	4.2	90
TM1720	Putative histone acetyltransferase (COG0454)	2.1	90
TM1777	NusA transcription elongation factor	2.5	90
TM1858	RecX protein, putative	1.9	90

Fig. 3 Clusters C1, C2, C3, and C4 were selected as responding to heat shock from a clustering of all genes. Time at which maximum fold change of expression with respect to the reference point (baseline at 80°C) and maximal fold change are reported. Columns of each cluster are chronological from B (baseline, 80°C) through 90 (90 min). Genes within clusters C1 and C2 were induced within minutes after temperature shift from 80°C to 90°C, while cluster C3 displayed maximal fold change late in heat shock. Genes in cluster 4 had above-average expression throughout the experiment and were induced upon heat shock. Fold changes were highly significant (all with $-\log_{10}$ *P*-value≥24)

(TM0747), and a hypothetical protein with homology to a protease maturation protein (TM1704) were induced twofold or more during the 90-min, heat-shock course. Both *T. maritima lon* protease genes (TM1633 and TM1869) and a bacteriocin homolog (TM0785) were downregulated during heat shock; a similar result for *lon* and a related bacteriocin homolog was noted for *Pyrococcus furiosus* under thermal stress at 105°C (Shockley et al. 2003). Lon proteases are currently classified in class IV of heat-shock proteins, most of which are regulated by unknown mechanisms (Versteeg et al. 2003).

SOS regulons have been characterized in both Gram-negative and Gram-positive bacteria (Koonin et al. 2000). The protein encoded by *TM1082* is related to both *E. coli* LexA (37%/198 aa) and *B. subtilis* DinR/LexA (38%/204 aa), which bind to different consensus sequences to repress their respective SOS regulons (Winterling et al. 1997). SOS regulon members are encoded in the *T. maritima* genome, including homologs to RecA (TM1859, 57% identity/330 aa with *B. subtilis* RecA) and RecX (Stohl et al. 2003) (TM1858, 27% identity/149 aa with *E. coli* RecX). UvrABD and RuvAB homologs are also identifiable by sequence similarity. A number of conserved SOS genes cluster together in cluster C4 of Fig. 3. Expression of the putative *lexA*/*dinR* repressor (TM1082) was downregulated by an average of 2.3-fold at 90°C. Although expression of *recA* recombinase homolog TM1859 was 1.9-fold higher after 90 min, this gene was highly expressed at baseline and throughout the time course, consistent with observations in biofilm and planktonic *T. maritima* populations (unpublished data). Induction of the *recA*-like gene *radA* in *P. furiosus* during heat

Table 1 Fold changes in expression of ATP-dependent proteases in *Thermotoga maritima*. Fold changes shown represent differences between each time point (0 min, 5 min, 10 min, 20 min, 30 min, 60 min, and 90 min) and the baseline (80°C); thus, the baseline is used as a reference condition here. Positive fold changes reflect up-regulation and negative fold changes reflect down-regulation

	Function	0 min	5 min	10 min	20 min	30 min	60 min	90 min
TM0146	ATP-dependent protease/ATPase ClpX	−1.2	−1.1*	1.1*	1.4	1.4	1.5	2.8
TM0198	ATP-dependent protease/ATPase ClpC-1	1.5	2.8	2.3	3.3	2.8	3.6	3.8
TM0521	Heat-shock protein HslV protease ClpQ	−1.3	−1.8	−1.2	−1.8	−1.7	−1.8	−1.3
TM0522	Heat-shock protein HslU ATPase ClpY	−1.2	−1.5	−1.3	−1.6	−1.6	−1.5	−1.1
TM0580	Cell-division protein FtsH	1.5	1.8	1.6	2.4	2.0	2.2	1.4
TM0695	ATP-dependent Clp protease, proteolytic subunit	1.1	−1.1*	−1.3	−1.3	−1.5	−1.5	−2.0
TM1391	ATP-dependent protease/ATPase ClpC-3	1.2	1.1*	−1.2	−1.4	−1.1*	1.1	1.0*
TM0873	ATP-dependent protease/ATPase ClpC-2 frameshift[a]	29.9	42.6	26.2	25.8	13.9	8.6	14.6
TM1633	ATP-dependent protease LonA	−1.2	−1.6	−1.5	−1.4	−1.2	−1.2	−1.1*
TM1869	ATP-dependent protease LonB, putative	1.0*	−1.2*	−1.1*	−1.6	−1.4	−1.3	1.0*
TM0145	Secreted metalloendopeptidase Gcp, putative	−1.3	−1.3	−1.3	1.0*	1.1*	1.1*	1.7
TM0409	Conserved hypothetical, probably secreted proteinase	−1.5	−1.8	−1.1*	−1.2	−1.3	−1.6	−1.7
TM0516	Clostripain-related protein	−1.1	−1.2	1.2	1.3	1.1*	1.0*	1.4
TM0571	Heat-shock serine protease, periplasmic (htrA)	1.1*	1.7	1.3	1.9	2.1	2.7	2.5
TM0643	Clostripain-related protein	−1.2*	−1.1*	−1.0*	1.1*	−1.4	−1.4	−1.9
TM0747	Carboxy-terminal protease	1.0*	1.3	1.3	1.8	1.7	1.9	2.5
TM0785	Protease, bacteriocin	1.1*	−1.6	−1.3	−2.5	−2.5	−3.1	−2.3
TM0890	Hypothetical zinc metalloprotease	−1.2	−1.3	1.0*	1.0*	1.1*	−1.2	1.1*
TM0916	Periplasmic serine protease, putative	−1.1	−1.2	−1.2	−1.2	−1.1*	1.2	−1.2
TM1346	Processing protease, putative	−1.3	−1.9	−1.5	−2.0	−1.8	−2.1	−3.0
TM1589	Clostripain-related protein	−1.8	−2.6	−2.2	−3.8	−3.7	−4.5	−4.3
TM1704	Hypothetical protein (homolog to protease maturation)	1.3	1.7	1.6	2.4	2.5	2.0	1.4
TM1822	FtsH protease activity modulator HflK	−1.2	−1.4	−1.2	1.1*	1.1*	1.1*	−2.1
TM1823	FtsH protease activity modulator HflC	−1.6	−2.1	−1.8	−2.0	−2.3	−2.3	−3.1

[a]TM0873 contains a known frameshift
*Not statistically significant as $-\log_{10}$ *P*-value is $\leq$ 5.4

Table 2 Fold changes in expression of sigma factor-related genes in *T. maritima*. Fold changes shown represent differences between each time point (0 min, 5 min, 10 min, 20 min, 30 min, 60 min, and 90 min) and the baseline (80°C); thus, the baseline is used as a reference condition here. Positive fold changes reflect up-regulation and negative fold changes reflect down-regulation. Sequence similarities were determined by BLAST search

	Function	0 min	5 min	10 min	20 min	30 min	60 min	90 min
TM0085	FlgM, putative regulator of flagellin synthesis	1.3	1.4	1.2	1.2	1.4	1.6	2.3
TM0534	σ^H, 33% identical/188 aa with *Bacillus subtilis* σ^H	1.0*	−1.1	1.0*	−1.2 *	−1.2*	−1.3	−1.9
TM0733	Putative anti-sigma factor, RsbW homolog	1.1*	1.8	1.9	2.8	1.8	1.7	1.1*
TM0902	σ^F, 33% identical/225 aa with *Escherichia coli* FliA/σ^F	−1.1*	−1.6	−2.2	−2.6	−2.1	−2.4	−2.7
TM1081	Putative anti-anti-sigma factor, SpoIIA homolog	−1.5	−1.9	−2.1	−1.9	−1.8	−2.2	−3.2
TM1354	Putative anti-sigma factor, RsbW homolog	−1.1*	−1.3	1.0*	−1.2	−1.2	−1.4	−1.3
TM1356	Putative positive regulator of σ^E, RseC homolog,	−1.2	−1.4	1.0*	1.0*	1.0*	−1.2	1.1
TM1442	Putative anti-anti-sigma factor, SpoIIA homolog	1.3	−1.1*	−1.3	1.0*	1.0*	1.2	−1.4
TM1451	σ^A, 53% identical/361 aa with *B. subtilis* σ^A	1.6	2.8	1.2	1.8	2.2	2.5	4.2
TM1598	σ^E, 69% identical/183 aa with *E. coli* σ^E	1.2	2.0	1.8	2.1	2.1	2.3	3.8

*Not statistically significant as $-\log_{10}$ *P*-value is $\leq$ 5.4

shock at 105°C has also been noted (Shockley et al. 2003). A *uvrB* homolog (TM1761) was maximally induced 4.6-fold over baseline at 90 min, while a *uvrD* homolog (TM1238) decreased 1.8-fold between 10 min and 90 min but remained highly expressed from baseline onward. An ATPase gene (*TM0508*) sharing sequence similarity with the ATPase domain of Holliday junction resolvases (COG2256), and the C-terminal domain of histone macroH2A1 (COG2110) also clustered closely with *uvrB*. The *T. maritima* genome contains genes encoding RuvA (TM0165) and RuvB (TM1730) homologs, which are involved in repair of Holliday junctions in *E. coli* (Dickman et al. 2002). The *ruvB* gene did not show significant changes during thermal stress but was highly expressed throughout all time points and clustered closest to *radA* (TM1859). Normalized expression levels of *ruvB*, *radA*, and *uvrB* at the 90-min time point were within the top 10% of all genes, with *ruvB* and *radA* within the top 5% of highly expressed genes at the 80°C baseline. High basal expression of these genes may indicate a temperature-dependent necessity for DNA damage repair in *T. maritima*. It has been suggested that the LexA binding site of Thermotogales might differ from Gram-negative and Gram-positive sequences due to the lack of obvious Cheo boxes upstream of the *lexA* homologs of *T. neapolitana* and *T. maritima* (Zverlov

Table 3 Fold changes in expression of other genes induced upon heat shock in *T. maritima*. Fold changes shown represent differences between each time point (0 min, 5 min, 10 min, 20 min, 30 min, 60 min, and 90 min) and the baseline (80°C); thus, the baseline is used as a reference condition here. Positive fold-changes reflect up-regulation and negative fold-changes reflect down-regulation. Genes in *bold* were up-regulated upon heat shock in *Bacillus subtilis*

	Function	0 min	5 min	10 min	20 min	30 min	60 min	90 min
TM0510	Iron-dependent transcriptional repressor, putative	1.6	1.9	1.8	2.1	2.2	2.8	4.6
TM0619	Conserved hypothetical protein, COG0438: Predicted glycosyltransferases RfaG	−1.6	−1.4*	−1.3*	−1.7	−1.6	−2.2	−1.6
TM0624	*N*-Acetylglucosaminyl-phosphatidylinositol biosynthesis-related protein	1.2	1.5	1.0*	1.0*	−1.1*	−1.8	−2.9
TM0630	Nucleotide sugar epimerase, putative	−1.5	−1.7	−1.6	−1.7	−1.8	−2.5	−3.5
TM0631	Lipopolysaccharide biosynthesis protein	−1.6	−1.5	−1.7	−2.1	−1.7	−1.7	−2.2
TM0668	Pleiotropic regulatory protein	−1.4	−2.0	−1.8	−2.0	−2.1	−2.3	−1.9
TM0689	Phosphoglycerate kinase, triose phosphate isomerase	−1.5	−1.9	−2.2	−1.5	−1.2	−1.2	−1.3
TM0694	Trigger factor, putative	−1.1*	−1.3	−2.8	−2.8	−2.3	−2.3	−2.2
TM0696	Ray-related protein	−1.5	−2.0	−1.5	−1.6	−2.1	−3.2	−4.0
TM0698	Flagellar biosynthesis protein FliP	−1.6	−1.9	−1.9	−1.9	−2.1	−3.2	−4.8
TM0700	Chemotaxis response regulator CheY	−1.2	−1.6	−1.6	−1.8	−1.7	−2.3	−2.2
TM0701	Purine-binding chemotaxis protein CheW	−1.4	−1.8	−1.9	−2.4	−2.2	−2.4	−2.2
TM0729	(p)ppGpp synthetase	1.2	1.3	1.6	1.6	1.7	1.7	2.5
TM0767	Maltodextrin glycosyltransferase	−1.3	−1.9	1.1	−1.9	−2.2	−3.1	−3.3
TM0807	Alkyl hydroperoxide reductase, putative	1.1*	1.4*	1.1	1.4	1.2*	2.3	2.1
TM0816	Transcriptional regulator, MarR family	1.6	5.1	3.0	9.3	21.7	76.8	108.0
TM0823	Transcriptional regulator, TetR family	1.2	4.6	2.0	8.0	8.3	12.4	19.0
TM0897	SpoVS-related protein	−1.1	−1.6	−1.5	−2.1	−2.1	−2.1	−2.0
TM0949	Transcriptional regulator, LacI family	−1.3	−1.4	−1.7	−1.8	−1.5	−1.4	−2.0
TM1005	Transcriptional regulator, putative, COG2207: AraC-type DNA-binding domain-containing proteins	1.2	1.6	2.1	2.8	3.4	3.9	4.5
TM1030	Transcriptional regulator, TetR family	1.2	3.9	3.6	5.5	4.7	5.9	7.5
TM1048	Endoglucanase	−1.3	−1.7	−1.3	−1.7	−1.7	−2.0	−1.9
TM1068	α-Glucosidase, putative	1.2	1.2	1.3	1.6	1.8	3.6	13.0
TM1069	Transcriptional regulator, GlpR/DeoR family	−1.1*	1.0*	1.1*	1.7	2.1	4.5	20.7
TM1082	LexA repressor	−1.5	−2.1	−2.7	−2.5	−2.3	−2.2	−2.5
TM1168	α-Glucan phosphorylase, authentic frameshift	1.2	−1.2	1.2	−1.5	−1.7	−2.9	−4.7
TM1176	Transcriptional regulator, metal-sensing	−2.5	−2.6	−2.0	−2.2	−2.1	−2.1	−1.9
TM1218	Transcriptional regulator, LacI family	−1.3	−1.6	−1.6	−2.1	−2.3	−2.7	−3.8
TM1219	Oligopeptide ABC transporter, ATP-binding protein	−1.4	−1.6	−1.3	−1.4	−2.0	−2.3	−3.4
TM1223	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein	−1.3	−1.9	−1.7	−1.7	−2.0	−1.8	−2.0
TM1232	Sugar ABC transporter, ATP-binding protein	2.9	3.2	3.2	4.5	3.4	2.6	2.9
TM1254	β-Phosphoglucomutase, putative	−3.0	−3.9	−2.5	−3.5	−3.1	−3.6	−2.7
TM1255	Aspartate aminotransferase	−1.4	−2.3	−1.5	−2.3	−1.9	−1.9	−1.5
TM1259	Phosphate regulon transcriptional regulatory protein PhoB	1.0*	−1.6	−1.8	−2.3	−2.7	−2.5	−3.3
TM1260	Phosphate transport system regulator PhoU	−1.1*	−1.9	−1.8	−2.5	−2.5	−2.9	−3.5
TM1275	Hypothetical protein	1.4	2.2	1.6	2.4	2.3	2.1	1.9
TM1276	Sugar ABC transporter, ATP-binding protein	2.6	3.4	2.8	3.6	2.9	3.2	3.2
TM1334	Conserved hypothetical protein, COG1032: Fe-S oxidoreductases family 2	−1.4	−1.7	−1.5	−1.7	−2.0	−2.6	−2.4
TM1363	Peptide chain release factor RF-1	−1.4	−2.1	−2.3	−2.3	−2.2	−2.2	−4.1
TM1368	ABC transporter, ATP-binding protein	1.1	1.5	1.8	2.5	2.2	2.4	2.7
TM1375	Spermidine/putrescine ABC transporter, periplasmic spermidine	1.0*	1.1*	1.3	1.1*	1.2*	2.5	3.8
TM1400	Aminotransferase	−2.6	−3.1	−1.9	−1.7	−1.2	1.1*	−1.7
TM1405	Lipopolysaccharide biosynthesis protein-related protein	1.0*	−1.2*	−1.5	−2.2	−2.0	−1.6	−1.8
TM1422	RnfB-related protein	1.1*	1.7	1.3	1.9	2.1	2.0	2.0
TM1436	Glycerol uptake operon antiterminator-related protein	1.1*	1.0*	1.0*	−1.3	−1.5	−2.4	−1.9
TM1450	Transcription repair coupling factor	−1.1	1.6	1.4	2.4	2.3	2.7	3.7
TM1468	Conserved hypothetical protein, COG1307: Uncharacterized BCR, DegV	−1.1*	−1.9	−1.8	−2.5	−2.9	−3.8	−3.3
TM1469	Glucokinase	1.2	−1.4	−1.2	−1.6	−1.9	−2.1	−2.5
TM1478	Methionine aminopeptidase	1.7	−1.4	−1.1*	−2.5	−2.4	−2.6	−3.5
TM1515	Ferric uptake regulation protein	−1.7	−1.9	1.8	−1.9	−1.9	−2.4	−2.3
TM1696	Type IV prepilin peptidase	−1.2	−1.3	−1.2*	−1.5	−1.6	−1.8	−2.3
TM1698	Aspartate aminotransferase	1.1	−1.2	−1.2	−1.3	−2.1	−3.0	−3.9
TM1706	Transcription elongation factor, GreA/GreB family	1.4	1.8	1.4	1.6	2.4	2.7	2.4
TM1707	Conserved hypothetical protein, COG1327: predicted transcriptional regulator, consists of a Zn-ribbon and ATP-cone domains	1.4	1.8	1.4	1.7	2.3	2.7	2.4
TM1719	DNA mismatch repair protein, MutS	1.1	1.7	1.9	2.6	2.6	2.8	3.1
TM1761	Excinuclease ABC, subunit B, UvrB	1.2	2.0	1.5	2.3	2.6	3.3	4.6
TM1776	Ferric uptake regulation protein	2.0	3.4	2.3	2.4	2.4	2.5	4.0
TM1834	α-Glucosidase	−1.1*	−1.6	−1.5	−1.9	−2.4	−2.8	−2.3

Table 3 (Continued)

	Function	0 min	5 min	10 min	20 min	30 min	60 min	90 min
TM1835	Cyclomaltodextrinase, putative	−1.3*	−1.5	−1.3*	−1.4	−2.2	−4.3	−4.3
TM1836	Maltose ABC transporter, permease protein	1.1*	−1.0*	−1.3	−1.5	−1.9	2.6	−2.7
TM1839	Maltose ABC transporter, periplasmic maltose-binding protein	1.1*	1.1*	1.0*	−1.2*	−1.5	−2.5	−2.7
TM1840	α-Amylase, AmyA	−1.3	−1.3	1.1*	−2.9	−3.3	−3.5	−2.4
TM1856	Transcriptional regulator, LacI family	1.1	1.5	1.6	1.7	1.6	2.3	2.4

*Not statistically significant as $-\log_{10}$ *P*-value is ≤ 5.4

and Schwarz 1999). However, sequences with high identity to the Gram-positive Cheo box consensus are found upstream of *recA* (Winterling et al. 1998) and a number of other genes with functions related to DNA damage repair in *T. maritima* (unpublished data). Further characterization of the *T. maritima* SOS regulon will undoubtedly yield insights into the details of DNA repair regulation at high temperatures.

Other identified stress response genes

In addition to known heat-shock genes, genes encoding several additional transcriptional regulators, including IclR/KdgR (TM0065), XylR (TM0032, TM0110), TetR (TM0823, TM1030), MarR (TM0816), LacI (TM0300, TM0949, TM1218, TM1856), GntR (TM0275) and GlpR/DeoR (TM1069) family proteins, showed significant expression changes during heat shock at 90°C (Fig. 3, cluster C4). TetR, MarR, and DeoR family transcriptional regulators were also induced in the *B. subtilis* thermal stress response (Helmann et al. 2001). The expression change of a MarR family protein (TM0816) (108.0-fold after 90 min at 90°C) was the most dramatic response observed during the time-course experiment, while a second MarR family protein (TM0710) showed little response to heat shock. *E. coli* MarR represses the *MarRAB* operon (Ariza et al. 1994; Cohen et al. 1993). It is deactivated by select antibiotic and anionic compounds, allowing the transcription of the AraC-family MarA, a transcriptional activator of multi-drug-resistance pump genes (Schumacher and Brennan 2002). While multiple AraC family proteins are present in *E. coli*, including MarA homologs Rob and SoxS, which also bind to MarA binding sites (Martin et al. 1999), TM1005 (Table 3) is the sole AraC family protein apparent in the *T. maritima* genome (COG2207). *T. maritima* MarA and MarR homologs appear to be distantly related to their *E. coli* counterparts. Whether the *T. maritima* MarA and MarR regulate processes similar to their mesophilic counterparts remains to be seen. The presence of a resistance-nodulation, cell-division-family exporter just two nucleotides downstream of the stop codon of TM0816 and multiple homologs to drug-exporter proteins within the *T. maritima* genome suggest potential regulatory targets for further exploration.

Differential expression of sigma factor-related genes

Regulation of stress response by various sigma factors has been well documented in several mesophilic bacterial species (Koonin et al. 2000; Yura et al. 2000). The targeted array used here contained all identifiable sigma, anti-sigma, and anti-anti-sigma factor genes in the *T. maritima* genome (Table 2). These included homologs to the genes encoding *E. coli* flagellar sigma factor FliA (TM0902, 35% identity/212 aa) (Helmann and Chamberlin 1987), *E. coli* heat shock and extracytoplasmic stress sigma factor σ^E (TM1598, 34% identity/183 aa) (Rouviere et al. 1995), and *B. subtilis* σ^H, a stationary phase sigma factor (TM0534, 30% identity/188aa) (Britton et al. 2002). A σ^A homolog (TM1451) is presumed to act as the vegetative sigma factor for *T. maritima*. This is because it is more closely related to *B. subtilis* σ^A (57% identity/263 aa) and *E. coli* σ^D(53% identity/272 aa) than to *E. coli*σ^H (28% identity/286 aa) or *B. subtilis* σ^B (23% identity/231 aa), both of which control well-documented heat-shock regulons (Yura and Nakahigashi 1999). Rapid induction of two of the four major sigma factor homologs was observed during heat shock: *sigA* (TM1451, 2.8-fold) and *rpoE* (TM1598, 2.0-fold) showed an early peak in expression 5 min after reaching 90°. *T. maritima* σ^A shares greater identity with the primary sigma factors of *B. subtilis* and *E. coli* than their corresponding heat-shock sigma factors; however, expression of *E. coli rpoD*has also been shown to be induced upon heat shock (Richmond et al. 1999). The lack of a close homolog to σ^H/σ^B in the *T. maritima* genome may indicate a simpler mode of heat-shock regulation operating in a smaller genome. Alternatively, it may reflect adaptation of this organism to the thermal variability of its natural habitat, where a heat-induced basal sigma factor may confer a survival advantage. An apparent lack of a major heat-shock sigma factor has also been observed for *Campylobacter jejuni* (Parkhill et al. 2000). Increased expression of an *rpoE* homolog upon heat shock suggests that σ^E may control expression of some genes important to the *T. maritima* heat-shock response as has been demonstrated for *E. coli* (Dartigalongue et al. 2001). In addition to roles in heat-shock response (Raina et al. 1995; Rouviere et al. 1995), σ^E homologs, such as *Pseudomonas aeruginosa* AlgU, have been implicated in virulence properties of pathogens (Raivio and Silhavy 2001). Differential expression of

*T. maritima sigA*and *rpoE* has also been observed in biofilm cells, suggesting a possible role for these sigma factors in directing the general stress response (unpublished data).

The *T. maritima sigH* gene (TM0534) showed little initial response to heat stress, but transcription decreased to a level 1.9 times lower than baseline by the 90-min time point. However, expression of the *fliA* sigma factor homolog TM0902 dropped 2.2-fold by the 10-min time point and remained lower than baseline for the remainder of the time course. Since homologs to the FlhCD (Arnosti 1990; Liu and Matsumura 1994) master regulators of class II flagellar genes and LrhA (Lehnen et al. 2002) have not been identified in *T. maritima*genome (Nelson et al. 1999), changes in the level of the FliA sigma factor may play an important role in regulating flagellar biosynthesis and export operons in this organism.

Conclusion

Examination of thermal stress response of the hyperthermophilic bacterium *T. maritima* showed that it is similar to less thermophilic bacteria in that transcriptional response to supraoptimal temperatures is rapid and widespread. Protein components of this heat-shock response resemble what has been discerned from studies of mesophilic bacteria, including apparent conservation of HrcA-CIRCE regulation of major heat-shock operons and induction of SOS-related genes. However, there are apparently differences with respect to regulatory strategies for sigma factors and ATP-dependent proteases and chaperones. The induction of the genes encoding *T. maritima* σ^{D}/σ^{A} and σ^{E}/σ^{W} homologs in response to heat shock suggests a mechanism for global regulation of *T. maritima* heat-shock genes in the absence of a major heat-shock sigma factor. Genetic, biochemical, and molecular analyses of specific genes and gene products will be needed to further explore the regulation of the *T. maritima* heat-shock response and determine the role of growth temperature in its evolution.

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