Extremophiles (2007) 11:495–503
DOI 10.1007/s00792-007-0063-4

ORIGINAL PAPER

Transcriptional responses of the deep-sea hyperthermophile *Methanocaldococcus jannaschii* under shifting extremes of temperature and pressure

Boonchai B. Boonyaratanakornkit · Li Yan Miao · Douglas S. Clark

Received: 17 October 2006 / Accepted: 26 December 2006 / Published online: 27 February 2007

Abstract Growth and transcriptional profiles of the deep-sea methanarchaeon *Methanocaldococcus jannaschii* were studied under sudden up-shifts of temperature and pressure. Application of 500 atm of hyperbaric pressure shifted the optimal growth temperature upwards by about 5°C in a high temperature–pressure bioreactor, and increased the specific growth rate threefold at 88°C. In contrast, pressure-shock from 7.8 to 500 atm over 15 min, the first such pressure up-shift reported for a piezophile, did not accelerate growth. High-pressure heat-shock from 88 to 98°C, a condition relevant to the turbulent in situ surroundings of deep-sea hydrothermal vents, resulted in termination of growth. Transcriptional profiles for cells grown at 88°C and 500 atm, heat-shocked at 500 atm, and pressure-shocked to 500 atm, shared a subset of genes whose differential expression was attributed to elevated pressure. In the pressure-shock case, this transcriptional response was evident despite the absence of a piezophilic growth response. In all, despite the piezophilic capacity and high-pressure origins of *M. jannaschii*, the core pressure response was remarkably limited and consisted of differential expression of genes encoding three hypothetical proteins and a gene involved in DNA recombination.

Communicated by K. Horikoshi.

B. B. Boonyaratanakornkit · L. Y. Miao · D. S. Clark (✉)
Department of Chemical Engineering,
University of California,
Berkeley, CA 94720-1462, USA
e-mail: clark@berkeley.edu

Keywords Barophiles · Deep-sea thermophiles · Methanogens · Piezophysiology · Piezophiles · High-pressure biosciences · Archaea

Introduction

It has generally been observed that deep-sea hyperthermophiles have optimal growth pressures above the pressure at the depth of their collection, while deep-sea psychrophiles have optimal growth pressures below their in situ pressure (Prieur 1997). This is the case for *Methanocaldococcus jannaschii*, which was isolated at a depth of 2600 m (corresponding to approximately 260 atm) (Jones et al. 1983), yet grew optimally at 86°C and 750 atm (1 atm = 0.1013 MPa) when hyperbaric helium pressure was varied from 7.8 to 750 atm in a high temperature–pressure (high *T–P*) bioreactor (Miller et al. 1988). Pressure also extends the maximum viable temperature of several deep-sea thermophilic archaea (Holden and Baross 1995; Canganella et al. 1997; Marteinsson et al. 1999a). However, a pressure of 250 atm did not extend the maximum growth temperature for *M. jannaschii* beyond 90°C, although the maximum temperature for methane production was extended to 98°C (Miller et al. 1988). The proportion of macrocyclic diphytanyl glycerol ether, a novel membrane lipid of *M. jannaschii*, was also shown to increase at 250 atm and 86°C (Kaneshiro and Clark 1995).

Whether thermophilic and psychrophilic piezophiles differ in their gene- and protein-expression response to pressure is currently unclear due to the limited data available. For example, Marteinsson and co-workers showed that when the deep-sea piezophilic hyperthermophile *Thermococcus barophilus* was grown at

400 atm, an unidentifiable protein was induced, while under atmospheric pressure, a stress protein was prominent (Marteinsson et al. 1999b). Similarly, Valle and co-workers reported a stress response at 0.1 MPa in *Photobacterium profundum* (Vezzi et al. 2005), a psychrophilic and slightly piezophilic bacterium isolated from 2,500 m (DeLong 1986). At 0.1 MPa, genes involved in protein folding, DNA repair, and amino acid transport were up-regulated (compared to 280 atm) (Vezzi et al. 2005). At 280 atm, genes in respiratory and fermentation pathways and genes involved in regulating polymer degradation were activated. However, there was no significant stress response for *Pyrococcus abyssi*, a hyperthermophilic archaeon originating from a depth of 2,600 m, at optimal or minimal growth temperatures as whole-cell protein profiles did not change at 30 atm compared to 400 atm (Marteinsson et al. 1997).

Recently, RNA expression for *M. jannaschii* was profiled to determine the effect of mass-transfer limitation at 500 atm and 88°C (Boonyaratanakornkit et al. 2006). Expression profiles from cells grown under mass-transfer limitation were compared to profiles from cells grown under conditions where dissolved gas-substrate concentrations did not limit growth. Under conditions where growth was limited by mass transfer of gaseous substrates, there was a stress response at both 7.8 and 500 atm. However, regardless of whether cells were mass-transfer limited, genes encoding a hypothetical protein, MJ0334, a replication protein A-related protein, MJ1159, and a gene involved in initiation of DNA replication, MJ0363, were up-regulated at 500 atm. At 500 atm, several genes that encode proteins involved in nitrogen metabolism were also differentially expressed.

To expand on these initial studies, here we report that *M. jannaschii* was exposed to lethal heat-shock at 7.8 and 500 atm and pressure-shocked from 7.8 to 500 atm with helium in a high *T–P* bioreactor. Lethal heat-shock of *M. jannaschii* at extreme pressures probably occurs around deep-sea hydrothermal vents, where temperatures range from above 350°C inside the vent to 2°C in the surrounding waters. Thus, high-pressure heat-shock experiments are relevant to the natural habitat of *M. jannaschii*. Because the transcriptional response to growth at 500 atm and 88°C was relatively limited (Boonyaratanakornkit et al. 2006), cells were rapidly up-shifted from 7.8 to 500 atm at 88°C for comparison. Although the organism does not experience rapid up-shifts in pressure in situ, these experiments provide a unique opportunity to compare transcriptional responses to two extreme environmental parameters of deep-sea hydrothermal vents. Furthermore, using a whole-genome cDNA microarray, we have explored adaptation of *M. jannaschii* under conditions mimicking the high pressures and severe temperature gradients that characterize deep-sea hydrothermal vents.

Materials and methods

Growth of *M. jannaschii* in the high temperature and pressure bioreactor

Under anaerobic conditions, *M. jannaschii* was grown in a SS316 high *T–P* bioreactor (High Pressure Equipment, Co.) with 200 ml of medium and at least 10% v/v inoculum as previously described (Park et al. 2006). First, the reactor was sparged with oxygen-free gas, and then the system was pressurized to 7.8 atm with H_2:CO_2 (4:1 v/v) gas substrate. To reach a pressure of 500 atm, helium was added to the gas substrate in the reactor by compression with an air-driven gas booster, thereby maintaining a constant partial pressure of H_2 and CO_2. Due to cell rupture upon sampling from 500 atm (Park and Clark 2002), the Bio-Rad protein assay reagent was used to measure the protein concentration and thereby track growth. For the lethal heat-shock experiments, the bioreactor was raised from 88 to 98°C in 1 h at both 7.8 and 500 atm. For the pressure-shock experiments, the pressure in the bioreactor was increased from 7.8 to 500 atm with helium at 88°C within 15 min. Four replicate experiments were performed under each condition (biological replicate samples). RNA samples from cells grown at 7.8 atm and 88°C were extracted in mid-exponential phase, RNA samples from cells lethally heat-shocked at 7.8 and 500 atm were extracted 0.5 and 1.5 h after the bioreactor reached 98°C, and RNA samples from pressure-shocked cells were extracted 0.5 and 1.5 h after the bioreactor reached 500 atm.

Array construction

Primers (Invitrogen) were designed for all 1,738 open reading frames (ORFs) using Primer 3 software. Polymerase chain reaction (PCR) was performed with 50 ng of genomic DNA, 0.8 μM of each forward and reverse primer, and 10 U of Taq DNA polymerase (New England Biolabs). Products were precipitated on 96 well plates with isopropanol and 3 M sodium acetate at 4°C and analyzed on a 0.8% agarose gel.

Poly-L-lysine SuperChip slides (Erie Scientific) were printed using a homemade linear servo arrayer

(http://www.derisilab.ucsf.edu/pdfs/MGuide3v2small.pdf). Dried PCR products were resuspended in 3× SSC and rearrayed in 384 well plates. Three copies of each ORF were printed in two arrays on each slide for a density of 6,018 PCR spots (total of 7,040 spots) on each array.

RNA preparation, labeling, and hybridization

Samples were withdrawn from the high *T–P* bioreactor through a micrometer control valve (High Pressure Equipment, Co.), passed through a 0.45 μm filter, placed into cold phenol with 1% SDS, and homogenized with a beadbeater as previously described (Boonyaratanakornkit et al. 2005). Total RNA was precipitated overnight with 3 M sodium acetate and isopropanol, treated with DNase I (Invitrogen), and cleaned with RNeasy columns (Qiagen). cDNA with aa-dUTP (Ambion) was synthesized from 2 μg of total RNA using random hexamers (Invitrogen) and SuperScript II (Invitrogen). NHS-Cy dye (Amersham Pharmacia) was coupled to the cDNA by incubation at room temperature for 1 h. Unincorporated dye was removed with QIAquick columns (Qiagen). Labeled cDNA was analyzed using a spectrophotometer, and targets with dye incorporation of over 10 pmol and nucleotides-per-dye ratios below 60 were used in hybridizations. These samples were resuspended in at least 10 μl of hybridization buffer (50% formamide, 5× SSC, 0.1% SDS, 1 μg μl^{-1} salmon sperm DNA) so that the dye concentrations in samples hybridized on the same slide were approximately the same.

Slides were prehybridized for 1 h at 42°C, washed with ultrapure H_2O and isopropanol, and blow-dried with filtered house air. A clean 22 mm × 25 mm glass lifterslip (Erie Scientific) was placed onto the prehybridized slide, and labeled cDNA target was applied using capillary action. Slides were placed in hybridization chambers (Corning) and hybridized at 42°C in the dark for 16–20 h. Post-hybridization washes were performed in glass dishes (VWR) on an orbital shaker with SSC buffer as previously described (Boonyaratanakornkit et al. 2005). Slides were blow-dried with filtered house air and scanned on a GenePix 4000 dual-color confocal laser scanner (Axon).

Twenty-four arrays were hybridized with the following pairs of samples: four biological-replicate pairs of samples extracted from 7.8 to 500 atm, both without heat-shock; four biological-replicate pairs extracted from 7.8 atm without heat-shock and from 7.8 atm 0.5 h after heat-shock; four biological-replicate pairs extracted from 7.8 atm without heat-shock and from 7.8 atm 1.5 h after heat-shock; four biological-replicate pairs extracted from 500 atm without heat-shock and from 500 atm 0.5 h after heat-shock; four biological-replicate pairs extracted from 500 atm without heat-shock and from 500 atm 1.5 h after heat-shock; and four biological-replicate pairs of samples extracted from 7.8 atm 0.5 h after heat-shock and from 500 atm 0.5 h after heat-shock. Each of the four pairs of biological replicates included two pairs of dye-swapped samples. Six arrays were hybridized with the following pairs of samples: three biological-replicate pairs of samples extracted from 7.8 atm without pressure-shock and from 500 atm 0.5 h after pressure-shock; and three biological-replicate pairs of samples extracted from 7.8 atm without pressure-shock and from 500 atm 1.5 h after pressure-shock.

Gene expression analysis

Array data were analyzed using the Bioconductor packages (http://www.bioconductor.org) within the R-gui environment (http://www.cran.r-project.org). Spots were identified and quantified using Spot (http://www.experimental.act.cmis.csiro.au/Spot/index.php). Non-normalized array data are available in the Gene Expression Omnibus database under the series accession number GSE4620. Data were lowess normalized within print-tip groups, scaled across print-tip groups and slides (to equalize the variances), and data from the three within-slide replicate spots and biological replicate samples were combined using the marray and limma packages (Bioconductor). Average expression ratios with corresponding Holm's adjusted *P*-values (Holm 1979) were calculated for cells exposed to pressure-shock or to temperature shock at 7.8 and 500 atm. Genes with adjusted *P*-values less than 0.05 were regarded as differentially expressed. The Holm's method adjusts for multiple testing and controls the family-wise (type 1) error rate. Therefore, genes for which the false positive rate (mean ratio of false positives to total rejections) was less than 0.05 were accepted.

A 2 × 2 factorial design (Glonek and Solomon 2004) was used to separate the interacting effects of pressure and lethal heat-shock on gene expression from the individual effects due to pressure or lethal heat-shock. For each gene g in array k, the log-base expression level, y_{kg}, is given by the following expression: $y_{kg} = \mu_g + \beta_{P,g}x_{1k} + \beta_{T,g}x_{2k} + \beta_{P:T,g}x_{1k}x_{2k} + \varepsilon_{kg}$, where μ_g is the baseline expression level of gene g at 7.8 atm without heat-shock, $\beta_{P,g}$ is the mean expression ratio of gene g at 500 atm relative to the baseline at 7.8 atm, $\beta_{T,g}$ is the mean expression ratio of gene g after heat-shock at 7.8 atm relative to the baseline at 7.8 atm

without heat-shock, x_{1k} and x_{2k} indicate the presence of 500 atm or heat-shock, respectively ($x_k = 1$ when condition is present and $x_k = 0$ when condition is not present), and ε_{kg} are independent errors with a mean of zero in the expression ratio for gene g relative to the baseline at 7.8 atm. Using the limma package (Bioconductor), $\beta_{P:T,g}$, the mean expression ratio of gene g due to the interaction of 500 atm and heat-shock, was determined using data from the 24 arrays described above. The experimental design for these 24 arrays was shown to give the smallest standard error for calculation of the interaction effect (Glonek and Solomon 2004). The interaction effect, $\beta_{P:T}$, for each gene was calculated by combining mean expression ratios as shown in Eq. 1:

$$\log_2^{500\ \mathrm{atm,TS}}(\text{fold change}) = \log_2^{7.8\ \mathrm{atm,TS}}(\text{fold change}) + \log_2^{500}(\text{fold change}) + \beta_{P:T} \quad (1)$$

where $\log_2^{500\ \mathrm{atm,TS}}$ (fold change) represents $\log_2$ of the fold change in gene expression occurring upon temperature shock (TS) at 500 atm relative to 7.8 atm without temperature shock.

Results

Pressure effects on growth rate

Under conditions where mass transfer of gaseous substrates did not limit growth, measurements at 7.8 and 500 atm and temperatures ranging from 58 to 90°C showed that growth was most accelerated by pressure at 88°C while there was only a small pressure effect at 58 and 90°C, temperatures near the growth limits for *M. jannaschii* (Fig. 1). At 88°C and 500 atm, the specific growth rate increased ca. threefold from 0.39 ± 0.03 h^{-1} at 7.8 atm to 1.2 ± 0.1 h^{-1}. Because of the large pressure acceleration at 88°C, the effects of 500 atm on gene expression were studied at this temperature.

Heat-shock responses at 7.8 and 500 atm

Methanocaldococcus jannaschii was lethally heat-shocked from 88 to 98°C at 7.8 and 500 atm (Fig. 2). Cells did not stop growing until ~1 h after the temperature set point was changed, which is about the time required for the reactor to reach 98°C. Expression profiles from cells lethally heat-shocked at 7.8 atm in the high *T–P* bioreactor were essentially the same as previously observed for cells lethally heat-shocked at 3 atm in serum bottles (Boonyaratanakornkit et al. 2005). The genes coding for the prefoldin α subunit, the thermosome subunit, the small heat-shock protein (sHSP), the hypothetical protein (MJ1463), and the CRISPR-associated protein (MJ0381) were all up-regulated by greater than twofold after heat-shock at 7.8 atm compared to at 7.8 atm without heat-shock (Table 1). In addition, several genes encoding transport proteins and genes associated with transcription were up-regulated, while genes coding for proteins involved in methanogenesis and flagellar synthesis were down-regulated.

The lethal heat-shock response at 500 atm exhibited similarities to the lethal heat-shock response at 7.8 atm (Table 1). However, several genes were differentially expressed after heat-shock at 500 atm but not after heat-shock at 7.8 atm, and these consisted of some of the same genes differentially-expressed at 500 atm

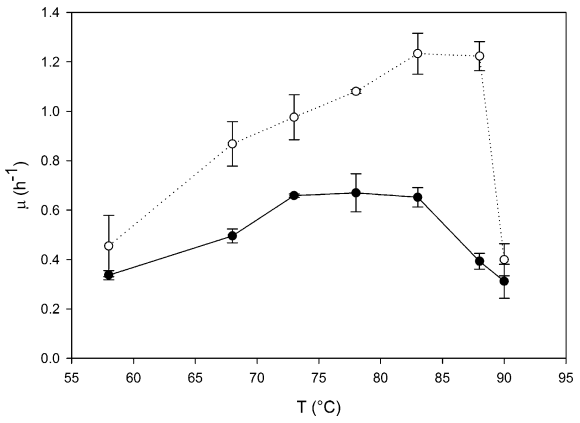

Fig. 1 Specific growth rates of *Methanocaldococcus jannaschii* as a function of temperature at either 7.8 atm (*closed circles*) or 500 atm (*open circles*)

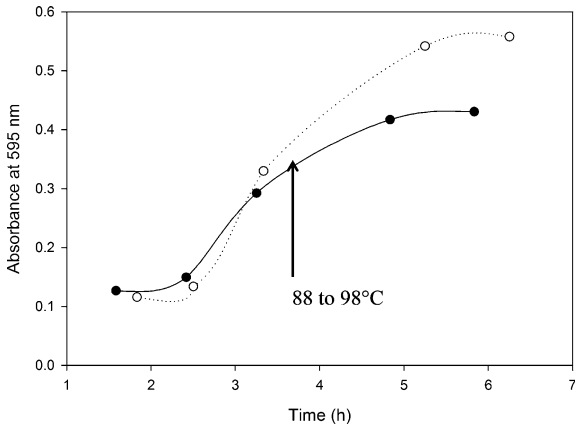

Fig. 2 Representative growth curves for heat-shock of *M. jannaschii* from 88 to 98°C at either 7.8 atm (*closed circles*) or 500 atm (*open circles*)

Table 1 Comparison of select differentially-expressed genes 0.5 h after the bioreactor reached 98°C when *Methanocaldococcus jannaschii* was heat-shocked from 88 to 98°C at 7.8 or 500 atm

ORF	Annotation	$\log_2^{7.8\ atm,TS}$ (FC)	$\log_2^{500\ atm,TS}$ (FC)
Chaperones and proteases			
MJ0648	Prefoldin α subunit, putative	5.6	4.9
MJ0999	Thermosome subunit	3.0	2.8
MJ0285	Small heat-shock protein	2.9	2.6
Transport proteins			
MJ1275	Na(+)/H(+) antiporter-related protein	2.3	3.6
MJ1015	Phosphate ABC transporter, periplasmic binding protein	1.6	NDE
MJ0566	Ferrous iron transport protein B	1.4	2.7
MJ1485	TRK system potassium uptake protein	1.2 (6.0E-3)	2.8
MJ0609	Transport protein	NDE	1.5
Amino acid and nucleotide biosynthesis			
MJ1274	Diphthine synthase (diphthamide = post-translational derivative of histidine)	NDE	2.4
MJ1096	*N*-acetyl-gamma-glutamyl-phosphate reductase (arginine biosynthesis from glutamate)	NDE	1.2
MJ1459	Adenine deaminase	NDE	1.4
Transcriptional regulators and transcription-related genes			
MJ0530	ZPR1-related zinc finger protein	3.6	2.9
MJ1545	Conserved hypothetical protein (with DNA binding domain)	2.4	2.1
MJ1220	Type I restriction-modification enzyme, M subunit, putative	2.0	2.4
MJ1164	Putative HTH-type transcriptional regulatory protein	1.6 (2.0E-3)	1.9 (1.0E-3)
MJ0012	Transposase-related protein	1.5	1.7
MJ1258	Archaeal histone A3	1.5	1.8
MJ0942	DEAH-box DNA helicase DinG, putative	1.3 (1.0E-3)	2.0
MJ0664	Hypothetical protein (topoisomerase I?)	1.2	1.2
MJ0168	Archaeal histone A1	1.1 (1.0E-3)	1.1 (4.2E-2)
MJ0171	DNA ligase	1.0	1.2
MJ0363	DNA replication protein, MCM family	NDE	1.0
MJ1159	Replication protein A-related protein	NDE	0.9 (5.0E-3)
Other			
MJ1463	UPF0128 family (only in thermophiles)	2.5	1.6 (1.0E-3)
MJ0381	CRISPR-associated negative autoregulator	1.4	1.1 (4.5E-2)
MJ0334	Hypothetical protein	NDE	1.2 (1.0E-3)
Methanogenesis			
MJ0852	*N*5-methyl-tetrahydromethanopterin: coenzyme M methyltransferase, subunit F	-1.0	–1.3
MJ0082	Methyl coenzyme M reductase II, subunit gamma	–1.0	–1.1
MJ0844	Methyl coenzyme M reductase I, operon protein C	–1.1	–1.2
MJ0083	Methyl coenzyme M reductase II, subunit alpha	–1.2	–1.2
MJ1035	*N*5,*N*10-methylene-tetrahydromethanopterin	–1.4	–1.4
Dehydrogenase			
MJ1194	Formylmethanofuran dehydrogenase (tungsten), subunit B, selenocysteine-containing	–2.7	–2.9
Flagellar synthesis			
MJ0902	Hypothetical protein (preflagellin peptidase?)	–1.1	–1.0
MJ0894	Flagella-related protein C, putative	–1.2	–1.3
Other			
MJ0656	Cytidylate kinase	NDE	–1.7
MJ1515	Conserved hypothetical protein (glutamine amidotransferase class-II?)	NDE	–0.9
MJ1336	Hypothetical protein (pfkB family carbohydrate kinase?)	NDE	–0.7 (1.0E-3)

P-values calculated using the Holm's step-down Bonferroni method and greater than or equal to 0.001 are shown in parenthesis. NDE (not differentially-expressed) means that the null hypothesis of no differentially-expression cannot be rejected based on the statistical cut-off of $P < 0.05$

FC fold change in gene expression

without heat-shock (Boonyaratanakornkit et al. 2006). For example, genes coding for a hypothetical protein, MJ0334, a replication protein A-related protein, MJ1159, and a gene involved in initiation of DNA replication, MJ0363, were up-regulated (Table 1). Genes encoding a conserved hypothetical protein that may be a class II glutamine amidotransferase (based on Pfam analysis), MJ1515, and a hypothetical protein,

MJ1336, were down-regulated. It should be noted that the responses observed 0.5 and 1.5 h after the bioreactor reached 98°C at either 7.8 or 500 atm were essentially the same (unpublished results).

Separation of pressure and heat-shock effects

We were able to separate the interacting effects of 500 atm and lethal heat-shock on gene expression from the individual effects due to growth at 500 atm or lethal heat-shock at 7.8 atm using a 2 × 2 factorial design (Yang and Speed 2002; Glonek and Solomon 2004). From Eq. 1 (Materials and methods), the interacting effect for each gene, $\beta_{P:T}$, can be calculated, since $\log_2^{500}$ (fold change) has previously been evaluated (Boonyaratanakornkit et al. 2006) and $\log_2^{7.8\ \mathrm{atm,TS}}$ (fold change) and $\log_2^{500\ \mathrm{atm,TS}}$ (fold change) have been determined in this paper. The interacting effect, $\beta_{P:T}$, for MJ0334 and MJ1515 were negative and positive, respectively (Table 2). Because MJ0334 was up-regulated at 500 atm without heat-shock, the negative value of the interacting effect for MJ0334 means heat-shock at 500 atm depressed the effect of pressure alone on induction of this gene (refer to Eq. 1). On the other hand, MJ1515 was down-regulated at 500 atm without heat-shock, and so the positive interacting effect for MJ1515 means that heat-shock at 500 atm depressed the effect of pressure alone on repression of this gene, e.g. MJ1515 was not as down-regulated when cells were heat-shocked at 500 atm.

In some cases, temperature shock effects were enhanced when heat-shock was induced at 500 atm compared to heat-shock at 7.8 atm. The interacting effect, $\beta_{P:T}$, was positive for a gene encoding a DNA helicase (MJ0942) and several genes encoding transport proteins (MJ1485, MJ0566, MJ0609), although the interacting effect was negative for one gene encoding a transport protein (MJ1015). As these genes were up-regulated upon heat-shock at 7.8 atm, the positive interacting effect for MJ0942, MJ1485, MJ0566, and MJ0609 means that heat-shock at 500 atm stimulated the effect of heat-shock alone on up-regulation of these genes.

In other cases, heat-shock at 500 atm resulted in the differential expression of genes for which heat-shock or elevated pressure alone did not significantly alter expression. The interacting effect for MJ0591, which encodes the α subunit of the proteasome, and MJ0987, which encodes the β subunit of the α/β prefoldin, a protein chaperone, were both negative. The negative interacting effect for both MJ0591 and MJ0987 indicates that heat-shock at 500 atm resulted in down-regulation of these genes relative to the expression level at 7.8 atm. In addition, the interacting effect for two genes involved in amino acid and nucleotide biosynthesis (MJ1274 and MJ1459) were positive, while the interacting effect for a gene involved in pyrimidine metabolism (MJ0656) was negative. Therefore, the interaction of heat-shock and elevated pressure appears to up-regulate MJ1274 and MJ1459 and down-regulate MJ0656 relative to the expression level at 7.8 atm.

Pressure-shock response

Interestingly, when cells were pressure-shocked from 7.8 to 500 atm over 15 min at 88°C, there was no lag in growth and pressure-accelerated growth was not

Table 2 Genes differentially-expressed by the interaction of 500 atm and heat-shock from 88 to 98°C 0.5 h after the bioreactor reached 98°C

ORF	Annotation	$\beta_{P:T}$
Transport proteins		
MJ1485	TRK system potassium uptake protein	2.1
MJ0566	Ferrous iron transport protein B	1.9
MJ0609	Transport protein	1.1
Other		
MJ0942	DEAH-box DNA helicase DinG, putative	1.6
MJ1274	Diphthine synthase (diphthamide = post-translational derivative of histidine)	1.5 (5.0E-3)
MJ1459	Adenine deaminase	1.0
MJ1515	Conserved hypothetical protein (glutamine amidotransferase class-II?)	1.0
Chaperones and proteases		
MJ0591	Proteasome, subunit alpha	–1.0
MJ0987	Prefoldin, subunit beta	–1.2
Other		
MJ0656	Cytidylate kinase	–1.3
MJ1015	Phosphate ABC transporter, periplasmic binding protein	–1.7
MJ0334	Hypothetical protein	–2.3

$\beta_{P:T}$ represents the log-base 2 expression change caused by interaction of 500 atm and heat-shock from 88 to 98°C that supplements the change caused by heat-shock from 88 to 98°C at 7.8 atm and the change caused by growth at 500 atm and 88°C (see Eq. 1). *P*-values calculated using the Holm's step-down Bonferroni method and greater than or equal to 0.001 are shown in parenthesis

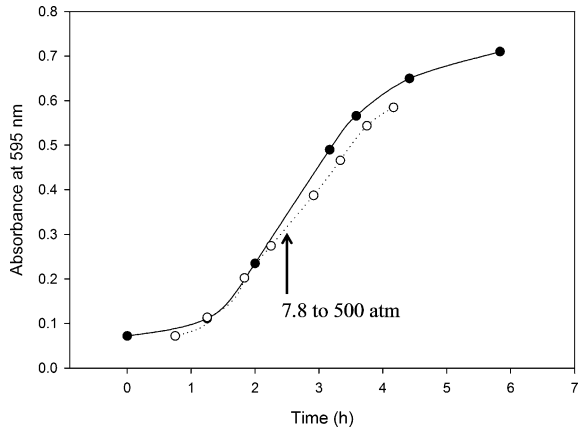

Fig. 3 Representative growth curves of *M. jannaschii* at 7.8 atm (*closed circles*) and with pressure-shock from 7.8 to 500 atm (*open circles*) at 88°C

observed (Fig. 3). Specific growth rates were $0.39 \pm 0.03\ h^{-1}$ at 7.8 atm and $0.40 \pm 0.12\ h^{-1}$ after pressure-shock to 500 atm. Even though pressure did not accelerate growth, pressure-shock intrinsically affected gene expression. As in the experiments at 500 atm and 88°C (Boonyaratanakornkit et al. 2006), MJ1159 and MJ0334 were both up-regulated while MJ1336 and MJ1515 were down-regulated relative to the expression level at 7.8 atm (Table 3). In addition, there was a stress response that is likely a consequence of the rapid pressure up-shift. Several genes coding for archaeal histones and a reverse gyrase that are involved in DNA supercoiling were up-regulated 0.5 and 1.5 h after pressure-shock. In addition, the gene encoding peptidyl-prolyl *cis/trans* isomerase (PPIase), MJ0825, a chaperone associated with the cold response, was up-regulated 0.5 h after pressure-shock. The gene coding for sHSP, MJ0285, a chaperone associated with the heat-shock response, was not up-regulated 0.5 h after pressure-shock but was up-regulated 1.5 h after pressure-shock.

Discussion

Growth rates for *M. jannaschii* were accelerated by pressure in a 1.15 L high *T–P* bioreactor as previously noted (Miller et al. 1988). When growth rates were measured between 58 and 90°C, application of 500 atm of hyperbaric pressure shifted the optimal growth temperature upwards by ~5°C. An upward shift in the optimal temperature was also observed in the hyperthermophilic piezophile, *Pyrococcus* strain ES4 (now known as *Pyrococcus endeavori*) (Pledger et al. 1994), although this is not the trend for all thermophilic piezophiles (Marteinsson et al. 1999a; Alain et al. 2002). Unlike *P. endeavori* (Pledger et al. 1994), the upper temperature limit for growth of *M. jannaschii* did not appear to be extended at 500 atm, a result also noted for *M. jannaschii* at 250 atm (Miller et al. 1988) and observed in other thermophilic piezophiles (Bernhardt et al. 1988; Alain et al. 2002).

Gene expression profiles of *M. jannaschii* under mass-transfer limitations at 500 atm (Boonyaratanakornkit et al. 2006), without mass-transfer limitations at 500 atm (Boonyaratanakornkit et al. 2006), temperature shocked at 500 atm, and pressure-shocked to

Table 3 Comparison of select differentially-expressed genes 0.5 h or 1.5 h after *M. jannaschii* was pressure-shocked from 7.8 to 500 atm at 88°C (designated by the superscripts PS0.5h and PS1.5h, respectively)

ORF	Annotation	$\log_2^{PS0.5h}$(FC)	$\log_2^{PS1.5h}$(FC)
Transcriptional regulators and transcription-related genes			
MJ1159	Replication protein A-related protein	1.6	1.4
MJ1512	Reverse gyrase, intein-containing (unique to hyperthermophilic bacteria/archaea)	1.1	1.5
MJECL17	Archaeal histone A5	1.1 (3.0E-3)	1.3
MJ1258	Archaeal histone A3	1.1 (1.9E-2)	1.7
Chaperones			
MJ0825	Peptidyl-prolyl cis-trans isomerase, FKBP-type rotamase, putative	1.0	NDE
MJ0285	Small heat-shock protein	NDE	1.4 (1.1E-2)
Other			
MJ0334	Hypothetical protein	NDE	1.2 (2.1E-2)
Other			
MJ1336	Hypothetical protein (pfkB family carbohydrate kinase?)	–1.2	–1.4
MJ1515	Conserved hypothetical protein (glutamine amidotransferase class-II?)	–1.4	–1.1

P-values calculated using the Holm's step-down Bonferroni method and greater than or equal to 0.001 are shown in parenthesis. NDE (not differentially-expressed) means that the null hypothesis of no differentially-expression cannot be rejected based on the statistical cut-off of $P < 0.05$

FC fold change in gene expression

500 atm all shared common characteristics. MJ0334, MJ1159, MJ1336, and MJ1515 were differentially-expressed under all of these conditions; thus, this response appears to be an intrinsic function of the elevated pressure. MJ0334 and MJ1336 encode hypothetical proteins with no homologs in any other species. MJ1515 codes for a conserved hypothetical protein that contains a domain characteristic of a glutamine amidotransferase class-II protein (Pfam). MJ1159 codes for a replication protein A-related protein that is involved in chromosomal replication and DNA recombination and repair (Kelly et al. 1998). MJ1159 may play a similar role as *recD* from *P. profundum*, which was hypothesized to limit DNA recombination at high pressure (Bidle and Bartlett 1999). Disruption of *recD* for *P. profundum* SS9 resulted in pressure-sensitive mutants, and insertion of the *P. profundum recD* into *E. coli* produced mutants displaying normal phenotypes at high pressures, i.e., no cell filamentation (Bidle and Bartlett 1999). It is also worth noting that the basic pressure response for *M. jannaschii* comprises a small number of genes compared to the heat-shock or cold-shock responses previously reported in this organism (Boonyaratanakornkit et al. 2005).

When lethal heat-shock was applied at 500 atm, the effect of pressure alone on differential expression of MJ0334 and MJ1515 was depressed. In addition, the expression of genes encoding transport proteins, genes which were already up-regulated upon heat-shock at 7.8 atm, was further enhanced. These genes were not up-regulated under constant temperature growth at 500 atm; therefore, the additional degree of upregulation appears to be an exacerbation of the heat-shock response at elevated pressures. Heat-shock at 500 atm also resulted in down-regulation of genes coding for the proteasome α subunit (MJ0591) and prefoldin β subunit (MJ0987) relative to the expression levels at 7.8 atm without heat-shock. Changes in the expression level of MJ0591 or MJ0987 were not observed under either constant temperature growth at 500 atm or after heat-shock at 7.8 atm. It is unclear why only the combination of heat-shock and 500 atm would result in depression of the expression levels of the chaperone and proteasome subunits. However, heat-shock at elevated pressure is a likely occurrence in the natural environment of *M. jannaschii*. Thus, these responses may underlie adaptation mechanisms of the hyperthermophile in its native habitat.

The pressure-shock experiment performed in this study is the first pressure-shock ever reported for a piezophile. Pressure-shock experiments have, however, been conducted for *E. coli* and *Saccharomyces cerevisiae*. In the case of *E. coli*, a pressure up-shift to 550 atm inhibited cell division and protein synthesis and resulted in cell filamentation and induction of several heat and cold-shock proteins (Welch et al. 1993). With *S. cerevisiae*, pressure-shock to 750 atm for 30 min induced a heat-shock protein, hsp104 (Tamura et al. 1998). When *M. jannaschii* was pressure-shocked to 500 atm in ca. 15 min, cells continued growing (based on protein concentration measurements), but the growth rate was not accelerated by pressure. The absence of pressure-accelerated growth may be related to the transcriptional stress response resulting from the rapid pressure up-shift, although it remains to be determined which particular elements of the stress response might prevent piezophilic growth.

Expression analysis indicated that there was an initial cold-shock response 0.5 h after pressure-shock, which was characterized by up-regulation of a gene encoding the cold chaperone, PPIase. PPIase catalyzes the isomerization of the peptidyl-prolyl bond, a protein-folding step that is often rate-limiting at low temperatures (Ideno and Maruyama 2002). Up-regulation of the PPIase-encoding gene could thus help compensate for any deleterious effects to protein folding induced by the pressure-shock. This pressure-shock response after 0.5 h transitioned to a heat-shock-like response 1.5 h after pressure-shock, characterized by up-regulation of the gene encoding the chaperone, sHSP. sHSP binds hydrophobic regions exposed by unfolded proteins, and may alleviate protein aggregation associated with the rapid up-shift from 7.8 to 500 atm. Interestingly, a heat- and cold-shock response was observed when *E. coli* was up-shifted to 550 atm (Welch et al. 1993), and this heat and cold response was also observed when *E. coli* was grown at 300 and 500 atm (Ishii et al. 2005). However, we are comparing a piezophilic archaeon to a non-piezophilic bacterium. While a heat-shock and cold-shock response was observed when *M. jannaschii* was pressure-shocked, no heat-shock or cold-shock response was observed when cells were cultivated at 500 atm.

That the same core pressure response was observed under growth, heat-shock, and pressure-shock at 500 atm suggests that the commonly affected genes are important for high-pressure adaptation. This core response does not appear to be a function of increased growth rate at 500 atm because pressure-shock to 500 atm did not result in accelerated growth. Furthermore, this core-pressure response was different from the response observed in the psychrophilic piezophile, *P. profundum* SS9 (Campanaro et al. 2005; Vezzi et al. 2005), in that there was no stress response induced at low pressure (7.8 atm) compared to at elevated pressure (500 atm). Lethal heat-shock of *M. jannaschii* at

500 atm resulted in a unique transcriptional response, which extended the effect of heat-shock at 7.8 atm while tempering the effect of pressure without heat-shock. In all, these results indicate that the complex and dynamic interplay between temperature and pressure in situ may exert additional effects beyond the surprisingly limited transcriptional response ascribed to pressure alone.

Acknowledgments We thank Dr. Chan Beum Park (Arizona State University) and the UC Berkeley Machine, Electric, and Glass Shops (College of Chemistry) for help in the design and construction of the high-pressure, high-temperature bioreactor system, Merrill Birkner (Statistics, UC Berkeley) for helpful comments on the manuscript, and Najib M. A. El-Sayed (TIGR), Anjana J. Simpson (TIGR), and Vivian Peng (Functional Genomics Lab, Berkeley) for assistance in the design and fabrication of the *M. jannaschii* cDNA array. This work was supported by the National Science Foundation (BES-0224733).

References

Alain K, Marteinsson VT, Miroshnichenko ML, Bonch-Osmolovskaya EA, Prieur D, Birrien JL (2002) *Marinitoga piezophila* sp. nov., a rod-shaped, thermo-piezophilic bacterium isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int J Syst Evol Microbiol 52:1331–1339

Bernhardt G, Jaenicke R, Ludemann HD, Konig H, Stetter KO (1988) High-pressure enhances the growth-rate of the thermophilic archaebacterium *Methanococcus thermolithotrophicus* without extending its temperature-range. Appl Environ Microbiol 54:1258–1261

Bidle KA, Bartlett DH (1999) RecD function is required for high-pressure growth of a deep-sea bacterium. J Bacteriol 181:2330–2337

Boonyaratanakornkit BB, Simpson AJ, Whitehead TA, Fraser CM, El-Sayed NMA, Clark DS (2005) Transcriptional profiling of the hyperthermophilic methanarchaeon *Methanococcus jannaschii* in response to lethal heat and non-lethal cold shock. Environ Microbiol 7:789–797

Boonyaratanakornkit BB, Cordova J, Park CB, Clark DS (2006) Pressure affects transcription profiles of *Methanococcus jannaschii* despite the absence of barophilic growth under gas-transfer limitation. Environ Microbiol 8:2031–2035

Campanaro S et al (2005) Laterally transferred elements and high pressure adaptation in *Photobacterium profundum* strains. BMC Genomics 6:122

Canganella F, Gonzalez JM, Yanagibayashi M, Kato C, Horikoshi K (1997) Pressure and temperature effects on growth and viability of the hyperthermophilic archaeon *Thermococcus peptonophilus*. Arch Microbiol 168:1–7

DeLong EF (1986) Adaptations of deep-sea bacteria to the abyssal environment (Thesis—PhD). In University of California, San Diego

Glonek GFV, Solomon PJ (2004) Factorial and time course designs for cDNA microarray experiments. Biostatistics 5:89–111

Holden JF, Baross JA (1995) Enhanced thermotolerance by hydrostatic-pressure in the deep-sea hyperthermophile *Pyrococcus* strain ES4. FEMS Microbiol Ecol 18:27–33

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Stat 6:65–70

Ideno A, Maruyama T (2002) Expression of long- and short-type FK506 binding proteins in hyperthermophilic archaea. Gene 292:57–63

Ishii A, Oshima T, Sato T, Nakasone K, Mori H, Kato C (2005) Analysis of hydrostatic pressure effects on transcription in *Escherichia coli* by DNA microarray procedure. Extremophiles 9:65–73

Jones WJ, Leigh JA, Mayer F, Woese CR, Wolfe RS (1983) *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch Microbiol 136:254–261

Kaneshiro SM, Clark DS (1995) Pressure effects on the composition and thermal-behavior of lipids from the deep-sea thermophile *Methanococcus jannaschii*. J Bacteriol 177:3668–3672

Kelly TJ, Simancek P, Brush GS (1998) Identification and characterization of a single-stranded DNA-binding protein from the archaeon *Methanococcus jannaschii*. Proc Natl Acad Sci USA 95:14634–14639

Marteinsson VT, Moulin P, Birrien JL, Gambacorta A, Vernet M, Prieur D (1997) Physiological responses to stress conditions and barophilic behavior of the hyperthermophilic vent archaeon *Pyrococcus abyssi*. Appl Environ Microbiol 63:1230–1236

Marteinsson VT et al (1999a) *Thermococcus barophilus* sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int J Syst Bacteriol 49(Pt 2):351–359

Marteinsson VT, Reysenbach AL, Birrien JL, Prieur D (1999b) A stress protein is induced in the deep-sea barophilic hyperthermophile *Thermococcus barophilus* when grown under atmospheric pressure. Extremophiles 3:277–282

Miller JF, Shah NN, Nelson CM, Ludlow JM, Clark DS (1988) Pressure and temperature effects on growth and methane production of the extreme thermophile *Methanococcus jannaschii*. Appl Environ Microbiol 54:3039–3042

Park CB, Clark DS (2002) Rupture of the cell envelope by decompression of the deep-sea methanogen *Methanococcus jannaschii*. Appl Environ Microbiol 68:1458–1463

Park CB, Boonyaratanakornkit BB, Clark DS (2006) Toward the large scale cultivation of hyperthermophiles at high-temperature and high-pressure. In: Rainey F, Oren A (eds) Methods Microbiol 35. Academic, New York

Pledger RJ, Crump BC, Baross JA (1994) A barophilic response by 2 hyperthermophilic, hydrothermal vent archaea—an upward shift in the optimal temperature and acceleration of growth-rate at supra-optimal temperatures by elevated pressure. FEMS Microbiol Ecol 14:233–241

Prieur D (1997) Microbiology of deep-sea hydrothermal vents. Trends Biotechnol 15:242–244

Tamura K, Miyashita M, Iwahashi H (1998) Stress tolerance of pressure-shocked *Saccharomyces cerevisiae*. Biotechnol Lett 20:1167–1169

Vezzi A et al (2005) Life at depth: *Photobacterium profundum* genome sequence and expression analysis. Science 307:1459–1461

Welch TJ, Farewell A, Neidhardt FC, Bartlett DH (1993) Stress response of *Escherichia coli* to elevated hydrostatic pressure. J Bacteriol 175:7170–7177

Yang YH, Speed T (2002) Design issues for cDNA microarray experiments. Nat Rev Genet 3:579–588