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Gene Expression Profile of *Helicobacter pylori* in Response to Growth Temperature Variation

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A *Helicobacter pylori* whole-genome DNA microarray was constructed to study expression profiles of *H. pylori* in response to a sudden temperature transfer from 37°C to 20°C. The expression level of the genome at each of four time points (15, 30, 60, and 120 min) after temperature downshift was compared with that just before cold treatment. Globally, 10.2% (n=167) of the total predicted *H. pylori* genes (n=1636) represented on the microarray were significantly differentially expressed ($p<0.05$) over a 120 min period after shift to low temperature. The expression profiles of the differentially expressed genes were grouped, and their expression patterns were validated by quantitative real-time PCR. Up-regulated genes mainly included genes involved in energy metabolism and substance metabolism, cellular processes, protein fate, ribosomal protein genes, and hypothetical protein genes, which indicate the compensational responses of *H. pylori* to temperature downshift. Those genes play important roles in adaption to temperature downshift of *H. pylori*. Down-regulation of DNA metabolism genes and cell envelope genes and cellular processes genes may reflect damaged functions under low temperature, which is unfavorable to bacterial infection and propagation. Overall, this time-course study provides new insights into the primary response of *H. pylori* to a sudden temperature downshift, which allow the bacteria to survive and adapt to the new host environment.

***Keywords*:** *Helicobacter pylori*, expression microarray, temperature

Helicobacter pylori is one of the world's most common pathogens and plays an essential role in the development of several acid-related and neoplastic gastroduodenal pathologies. Over twenty years after Marshall and Warren cultured *H. pylori*, we are still uncertain about exact mode of transmission and source of *H. pylori* infection. Molecular methods have detected the presence of *H. pylori* DNA in river water, well water, and waste water, as well as in surface and shallow groundwater, suggesting that this organism is waterborne and may be transmitted by the fecal-oral route (Hegarty *et al*., 1999; Moreno *et al*., 2003). *H. pylori* has an optimal growth temperature of 37°C but likely to encounter a wide range of temperatures during the contamination cycle. It must, therefore, be able to sense, adapt, and respond to these temperature fluctuations. Temperature change is the most common stressor encountered by all living organisms in natural habitats. Differential gene expression at different temperature may allow *H. pylori* to colonize its host efficiently, leading to commensalism or pathogenesis. The accumulated evidence suggests that bacteria exhibit complex physiological responses to a rapid decline in temperature (Gualerzi *et al*., 2003; Phadtare and Inouye, 2004). A better understanding of the physiological response of *H. pylori* to low temperatures is essential for predicting microbial growth and pathogenetic mechanisms at these temperatures.

However, the molecular basis underlying the ability of *H. pylori* to survive and adapt to various environmentally relevant stressors is poorly understood. With the availability of whole genome sequences and DNA microarrays, the global response to temperature decrease has been studied in diverse bacteria at the transcriptional level. Recently, we constructed a DNA microarray by PCR amplification of unique fragments of each open reading frame (ORF) from the annotated genomic sequence of *H. pylori* 26695 and J99 (Han *et al*., 2007). This microarray consists of 1882 DNA fragments, corresponding to 97.3% (1636/1681) of the ORFs of both sequenced *H. pylori* strains, of which 1549 ORFs belong to *H. pylori* 26695 and 87 belong to *H. pylori* J99. To define this organism's molecular response to decreased growth temperature, temporal gene expression profiles were examined in cells subjected to cold stress by using whole-genome DNA microarrays for *H. pylori*.

Materials and Methods

Bacterial strain and culture conditions

Plate cultures of *H. pylori* SS1 (Sydney Strain) were grown in selective Columbia agar (Oxoid, USA) supplemented with 7% defibrinated horse blood and Skirrow selective antibiotics (5 mg/ml trimethoprim, 10 mg/ml vancomycin, 2500 U/ml

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polymyxin B, Sigma, USA). Plates were placed under microaerobic conditions (N_2 85%, CO_2 10%, O_2 5%) and incubated at 37°C for 72 h. For exposure to low temperature conditions, *H. pylori* was grown in brain heart infusion broth (BHIB) containing 7% fetal bovine serum (FBS) and Skirrow selective antibiotics with shaking in a microaerobic environment. Bacterial viability and morphological distribution were determined by colony morphology, Gram staining and positive urease, catalase, and oxidase. At certain intervals, bacterial samples in liquid media were collected to determine the optical density at 600 nm (OD_{600}) and colony forming unit (CFU) by plating on agar plates. A bacterial growth curve was determined.

Temperature variation and RNA isolation

An 72 h liquid culture of *H. pylori* SS1 was harvested by centrifugation (5 min, 5,000 rpm), and resuspended to an OD_{600} of 0.08 to 0.1 in fresh Brucella broth supplemented with 10% FBS. The master culture was then split equally to yield two independent cultures. The cultures were maintained under microaerobic conditions with shaking on a rotary platform (250 rpm) at 37°C, then were transferred to 20°C at mid-log phase (OD_{600} of 0.45∼0.50) according to the mapped growth curve. Reference samples were harvested immediately prior to the transfer and were represented as T_0 time point. Test samples were collected at 15, 30, 60, and 120 min after the temperature downshift and were represented as T_1, T_2, T_3, and T_4, respectively. For each time point, an aliquot was removed for OD_{600} measurement, CFU count, and microscopic visualization of the culture for assessment of motility and morphology. Cells were immediately collected by centrifugation at 4°C (3 min, 5,000 rpm), resuspended in 2 ml Trizol, then placed at -80°C until use. *H. pylori* RNA was isolated using TRIzol reagent (Gibco/BRL) as previously described (Thompson *et al.*, 2003), and purified using RNeasy Mini kit (QIAGEN, Germany) and DNase digestion. Two individual experiments of *H. pylori* growth in broth culture were performed. These were performed on different days and used individual cultures for each time point. The RNA concentration was quantified using a spectrophotometer, and RNA integrity was monitored by agarose gel electrophoresis.

Table 1. Primers used in the real-time PCR experiment

Gene ID or name	Primer sequence (5'-3')	Gene product length (bp)
16S rRNA	F: AGCGTTACTCGGAATCACTGG R: ATTCCACCTACCTCTCCCACA	131
HP0084	F: CAAAATCCCCACAATCCACAT R: TTTTAGACGCCAAAGACAAGG	111
HP0286	F: CGATGAGGTGGTGGTGATAGG R: CGGTAAAGAAATGAAAATGCG	103
HP0561	F: TCAAAAGAAACGCTACCGCT R: TTTCAACTCTGTAACGCCCG	146
HP1050	F: GTGCTCATTAGGGCGTTATTTTC R: CGTTGTTGCTCTAAAGACAGGAT	107
HP1108	F: CGTTCGCTTCTTATGGTTCAG R: ACCAGGGTCAATCACCAGCAC	128
HP1227	F: AAGGTTATTATGGCTTTAGGCG R: ATGTTGACGATTTTGCTTTTGC	143
HP1430	F: TGCTCATAATCACCCTCCCTT R: TCCACCAACTCCCATAAATCC	103

Preparation and hybridization of cDNA probes

Each test sample (T_1, T_2, T_3, and T_4) was labeled and hybridized on separate *H. pylori* microarrays together with reference sample (T_0). cDNA was synthesized from 10 μg each of test RNA and reference RNA in a standard reverse transcriptase reaction using Superscript II (Invitrogen) and random nanomer (Sigma Genosys). Synthesized cDNA was purified using QIAquick PCR purification columns (QIAGEN) as specified by the manufacturer and subsequently labeled with Cy5 (reference RNA, green) and Cy3 (test sample, red) fluorophores, as previously described (Thompson *et al*., 2003). Individual Cy5 and Cy3 reactions were combined, and unincorporated dye was removed using QIAquick Nucleotide Removal kit (QIAGEN) as specified by the manufacturer. The eluates from the columns were concentrated by evapo-

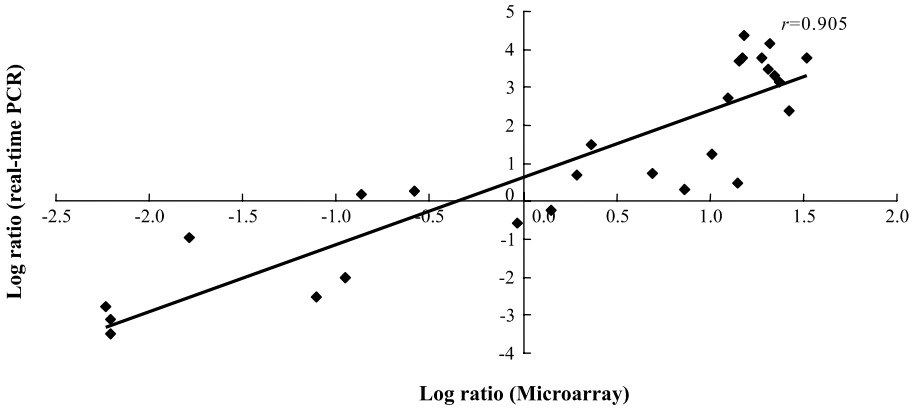

Fig. 1. Comparison of gene expression measurements by microarray and RT-PCR assays. The relative transcriptional levels for 7 genes listed in Table 1 at four time points were determined by microarray and real-time RT-PCR. The real-time RT-PCR log2 values were plotted against the microarray data log2 values. The correlation coefficient (*r*) for comparison of the two datasets is 0.905.

ration in a Speed Vac and resuspended in 100 μl of EB buffer. The labeled DNA sample was combined with 11 μg salmon sperm DNA, heated to 70°C for 45 min, cooled briefly, and then added to 7.5 μl 20× SSC, 0.3 μl 10% SDS, and 20 μl formamide. The mix was added to the *H. pylori* microarray for hybridization at 37°C for 16 h, and stringency washes were performed as previously described (Salama *et al*., 2000). Each hybridization experiment was repeated twice using the total RNA isolated from two independent time course experiments.

Data analysis

After hybridization, microarray was scanned and analyzed using Tiffsplit (Agilent) to calculate the signal intensities and to determine the presence or absence of each ORF. Spots were excluded from analysis due to obvious spot abnormality and low signal (signal/noise<2.0). The data obtained for the net pixel intensity in each microarray channel were normalized and clustered using Genespring 4.0. Student's t test/analysis of variance was used to compare the mean expression levels of the test and reference samples. The ratio of the red (test sample) to green (reference sample) channels for each spot was expressed as R/G ratio. Genes with significant differential expression levels ($p<0.05$) were selected. Biologically independent experiments were analyzed separately. Genes with an expression ratio greater than or equal to 2.0 were regarded as up-regulated, with those less than or equal to 0.5 were regarded as down-regulated, even though a 1.5-fold change in relative transcript abundance can be biologically significant (Smoot *et al*., 2001; Stintzi, 2003). Data obtained from each time course were assessed separately and only the genes which showed consistent expression patterns between experiments were selected.

Assessment of array data quality

Microarray results were further validated by quantitative real-time PCR (RT-PCR) using an ABI Prism 7700 DNA analyzer (Applied Biosystems, USA) and the QuantiTect SYBR green RT-PCR kit according to the manufacturer's protocol. Seven genes exhibiting induced, unchanged, and repressed expression at four time point were selected for RT-PCR analysis with the same RNA samples used in the array hybridizations. Primers were designed using Primer Premier 5.0 software (Table 1). The reaction was performed in 40 cycles of 10 min at 95°C, 10 sec at 95°C, and 1.5 min at 56°C~59°C. The gene expression levels obtained by RT-PCR analysis were normalized to that of the 16S rRNA gene because its expression is invariant under different temperature changes. Quantitative values were obtained using the comparative threshold cycle (C_t) method recommended by Applied Biosystems (Stintzi, 2003). The resulting gene expression ratio was log transformed and plotted against the average log ratio values obtained by microarray analysis (Fig. 1).

Results and Discuss

***H. pylori* growth at different time point**

Microscopic observations revealed that bacterial morphology was almost identical at each time point (T_0, T_1, T_2, T_3, and T_4). OD_{600} and CFU of *H. pylori* SS1 cultures at each time point were proximal. This suggested that the observed transcriptional profiles were not influenced by growth phase or cell density difference of the two temperature condition.

Reliability of array data

The reliability of the microarray data was assessed by cohybridization of two cDNA samples prepared from the same total cellular RNA. Seven genes with different expressions were chosen. The expression of HP0084, HP1108, HP1227, and HP0561 were greatly induced, the expression of HP1050 and HP1430 were repressed, and the expression of HP0286 did not change (Tables 2 and 3). A high level of concordance ($r=0.905$) was observed between microarray and RT-PCR data despite quantitative differences in the level of change. Consequently, the RT-PCR results corroborated the microarray results.

Global gene expression analysis

In total, 10.2% (n=167) of the total predicted *H. pylori* genes (n=1636) represented on the microarray were significantly differentially expressed ($p<0.05$) after transfer to low temperature at least once. Of the total predicted genes, 5.7% (93/1636) genes were up-regulated and 4.5% (74/1636) were down-regulated (Fig. 2). Up-regulated genes included hypothetical protein genes (46/93, 49.5%) and substance metabolism genes (including protein, nucleic acid, cofactors, and carriers; fatty acid and phospholipids biosynthesis; central intermediary metabolism) (11/93, 11.8%), energy metabolism genes (9/93, 9.7%), cellular processes genes (7/93, 7.5%), protein fate genes (6/93, 6.5%) and ribosomal protein genes (7/93, 7.5%) (Table 2). Down-regulated genes included hypothetical protein genes (35/74, 47.3%) and DNA metabolism genes (11/74, 14.9%), cell envelope genes (6/74, 8.1%) and cellular processes genes (6/74, 8.1%) (Table 3). This indicated that temperature downshift led to drastic changes in the physical metabolism of *H. pylori*. To overcome the deleterious effects of cold shock and to ensure that cellular activity will resume or be maintained at low temperature, bacteria have developed a transient adaptive response, the cold shock response, during which the level of a specific group of proteins increases (Derzelle *et al*., 2000). It is clear that the essential nature of a gene is dependent on the environmental conditions, because there are many genes essential under some conditions but not others (Schilling *et al*., 2002). The cold shock response can protect bacterial cellular components, such as DNA, cellular membranes, ribosomes, and can help avoid functional obstacle. Many genes whose expression were altered by temperature downshift encoded for proteins of unknown function. This displays the limits of our understanding of *H. pylori* physiology, which may have essential function in *H. pylori* pathogenesis.

One of the remarkable findings observed in this time course microarray analysis was that the global change in gene expression upon temperature downshift was relatively early and persistent (Fig. 3). 38.7% (36/93) of up-regulated gene expression occurred 15 min after transfer to 20°C. Of these, 88.9% (32/36) remained after 120 min. 39.8% (37/93) of the up-regulated genes displayed 30 min after temperature decrease. Of these, 72.9% (27/37) remained after 120 min. Only 16.1% (15/93) and 5.4% (5/93) of up-regulated

gene expression occurred at 60 min and 120 min, respectively, after temperature transfer. 37.8% (28/74) of down-regulated gene expression occurred 15 min after transfer to 20°C. Of these, 82.1% (23/28) remained after 120 min. 27% (20/74) of down-regulated genes displayed 30 min after temperature decrease. Of these, 80% (16/20) remained after 120 min. 8.1% (6/74) and 27% (20/74) genes were down-regulated 60 min and 120 min, respectively, after transfer to 20°C. These initial global changes in transcription within 30 min reflected the primary response of *H. pylori* to surmount the sudden temperature decrease, thereby allowing the bacterium to survive and ultimately to adapt to the new temperature. As exemplified here, time course experiments provide detailed insight into the mechanistic responses to stresses and are essential for deciphering the mechanism of bacterial response and adaptation to stressors, which is difficult to elucidate or identify using single time point studies (Stintzi, 2003).

Classification of temperature-related genes

Heat-shock protein genes

Our observations are based on shifting cultures from 37°C to 20°C, which simulates conditions that would be encountered during the transmission course of *H. pylori* infection. Heat-shock proteins help the cell to refold temperature-damaged proteins via chaperones and to prevent aggregation and misfolding of intracellular proteins under stress conditions (Arsene *et al*., 2000; Schumann *et al*., 2002). In bacteria, the major molecular chaperones include the DnaK machine and the GroE machine (GroES and GroEL). The heat shock proteins of *H. pylori* may play a role in the protection of the bacteria from hostile environments and in specific pathogenic processes (Homuth *et al*., 2000).

Increased expression of *H. pylori groES* (HP0010), *groEL* (HP0011), *dnaK* (HP0109), *grpE* (HP0110) were detected in our study, which were expected to be down-regulated at low temperatures (Beckering *et al*., 2002; Han *et al*., 2005). But recently, an increasing number of reports suggest that heat shock proteins can be induced by cold shock, and that the proper folding of proteins, as well as the refolding of cold-damaged proteins, is important for bacterial growth during cold shock (Phadtare *et al*., 2004; Crapoulet *et al*., 2006; Lee *et al*., 2008). Under physiological conditions, DnaK represents the intracellular stress sensor. The *dnaK*-null mutant of *E. coli* lacks the ability to grow at 15°C (Yoshimune *et al*., 2005). *C. crescentus* strain SG400 cells lacking DnaK/J are more sensitive to freezing, exhibit decreased viability (80%) after incubation for 1 h at -80°C, which indicates that the absence of DnaK/J results in a decrease in the ability of *C. crescentus* cells to survive in freezing temperatures (Susin *et al*., 2006). The up-regulation of these heat shock protein genes indicate that they play important roles in adaption to temperature downshift in *H. pylori*.

Energy metabolism genes

H. pylori can grow under both aerobic and anaerobic conditions. Homologues of enzymes involved in the utilization of carbohydrates and enzymes of the Entner Doudoroff and pentose phosphate pathways are present in the *H. pylori* genome, suggesting that glucose can be used as a source of energy. Our study revealed that most genes involved in energy metabolism of *H. pylori* were up-regulated, which was consistent to other studies (Crapoulet *et al.*, 2006). The increased levels of energy generation indicate that the cells incubated at low temperature are stressed by energy limitation. Up-regulation of energy metabolism genes may reflect the energy-starved condition of the cell and the necessity for saving and reallocating energy for repairing damages caused by the temperature downshift.

Ribosome genes

Ribosomes are thought of as sensors for the heat and cold shock response networks in bacteria, and are involved as signals linking the environmental stimulus (temperature) with the increased expression of heat shock genes (VanBogelen and Neidhardt, 1990). We found that a number of genes encoding ribosome proteins showed higher transcript levels at 20°C, including HP0296, HP0297, HP0562, HP0084, HP1151, HP1319, and HP1320. This result is consistent with the fact that ribosome proteins may be important for the correct assembly of rRNA at low temperatures (Graumann *et al*., 1996). In *E. coli*, some ribosomal protein genes (*rpmB*, *rplT*, *rpsN*, and *rplP*) are also up-regulated, which suggests temperature downshifts induce the stabilization of secondary structures of nucleic acids (Jones *et al*., 1992). Other ribosome protein genes, including HP1301, HP1305, and HP1307, were down-regulated to the same extent. The reason for such antagonistic regulation was unclear.

Restriction-modification (R-M) systems

In our study, the transcription of most R-M genes was repressed by cold treatment. Only HP1369 was induced. The majority of the *H. pylori* R-M systems are of type II. Type II systems consist of two separate enzymes: a restriction endonuclease, which is responsible for the breakdown of foreign DNA, and a modification DNA methyltransferase (methylase), which protects endogenous DNA from endonucleolytic digestion by methylating them at the endonuclease recognition sites. R-M systems may be implicated in cellular defense and in many critical other cellular processes, including transcriptional regulation, initiation of DNA replication, and genomic imprinting (Srikhanta *et al*., 2005). The presence of R-M systems in *H. pylori* has been proposed to be associated with the bacterial ability to infect its host (Bjorkholm *et al*., 2002). The down-regulation of *H. pylori* R-M genes indicates damaged function under low temperature, which is unfavorable to its self-protection and infection. It will be interesting to conduct further studies on their regulation mechanisms at low temperatures.

Outer membrane protein genes

Colonization by *H. pylori* involves an interaction between the outer membrane of the bacterium and the gastric epithelium of the host. The unusual set of outer membrane proteins and the specialized outer membrane of *H. pylori* are consistent with its persistence in a restricted niche. Outer membrane proteins, such as HP0913, may play an important role in the colonization or persistence of *H. pylori* infection or in the severity of disease associated with its infection (de Jonge *et al*., 2004). Furthermore, many outer membrane proteins play

Table 2. Genes induced upon temperature downshift in *H. pylori*

Gene ID	Gene functionand classification[a]	Fold change[b]			
		15 min	30 min	60 min	120 min
Protein fate					
HP0010	chaperone and heat shock protein	2.674	2.501	2.758	2.766
HP0011	co-chaperone	2.897	2.876	2.672	2.937
HP0109	chaperone and heat shock protein 70	2.801	2.638	2.701	2.728
HP0110	co-chaperone and heat shock protein	3.072	2.972	2.878	2.997
HP1255	protein translocation protein, low temperature	1.983	2.936	3.178	2.566
HP0382	zinc-metallo protease	2.016	2.606	2.441	2.277
Cellular processes-Chemotaxis and motility					
HP0103	methyl-accepting chemotaxis protein	1.469	1.416	2.034	2.104
HP1192	secreted protein involved in flagellar motility	2.171	2.692	2.464	2.394
HP0325	flagellar basal-body L-ring protein	1.549	2.063	2.393	2.669
HP0684	flagellar biosynthesis protein, authentic frameshift	1.628	1.962	2.408	3.403
HP0243	neutrophil activating protein (*nap*A)	2.656	2.668	2.024	2.091
HP0538	*cag* pathogenicity island protein	1.493	1.898	2.002	2.376
HP1496	general stress protein	2.090	2.812	2.866	2.737
Energy metabolism					
HP0588	ferrodoxin-like protein	1.770	2.366	2.176	2.068
HP0632	quinone-reactive Ni/Fe hydrogenase, large subunit	1.818	1.750	2.020	1.616
HP0633	quinone-reactive Ni/Fe hydrogenase, cytochrome b subunit	1.944	2.308	2.317	1.945
HP0642	NAD(P)H-flavin oxidoreductase	2.017	2.071	1.874	1.936
HP1227	cytochrome c553	2.495	2.862	2.837	2.236
HP1099	2-keto-3-deoxy-6-phosphogluconatealdolase	1.931	2.212	1.589	1.748
HP1108	pyruvate ferredoxin oxidoreductase, gamma subunit	1.843	2.217	2.252	2.265
HP1260	NADH-ubiquinone oxidoreductase, NQO7 subunit	1.195	1.790	2.350	2.011
HP1262	NADH-ubiquinone oxidoreductase, NQO5 subunit	1.379	1.686	2.005	2.144
Cell envelope					
HP1125	peptidoglycan associated lipoprotein precursor	1.587	1.897	2.186	2.196
HP0227	outer membrane protein	2.945	2.732	2.755	2.837
HP0229	outer membrane protein	2.211	1.561	1.445	1.445
Protein synthesis					
HP0835	histone-like DNA-binding protein	2.171	2.535	2.508	2.293
HP1256	ribosome releasing factor	2.341	2.992	2.854	2.252
HP0827	ss-DNA binding protein 12RNP2 precursor	1.823	2.816	2.616	2.607
Ribosomal protein					
HP0296	ribosomal protein L21	4.124	7.406	6.653	7.416
HP0297	ribosomal protein L27	3.385	4.817	3.968	4.384
HP0562	ribosomal protein S21	1.822	2.539	2.338	2.513
HP0084	ribosomal protein L13	2.135	2.591	2.536	2.688
HP1151	ribosomal protein S16	1.622	2.196	2.55	2.664
HP1319	ribosomal protein L3	2.006	2.694	2.817	2.547
HP1320	ribosomal protein S10	1.848	2.393	2.746	2.306
Purines, pyrimidines, nucleosides, and nucleotides					
HP0919	carbamoyl-phosphate synthase (glutamine-hydrolysing)	1.712	2.099	2.218	2.072
HP1178	purine-nucleoside phosphorylase	2.280	2.327	2.456	2.066
HP1257	orotate phosphoribosyltransferase	1.635	2.025	2.28	1.823
HP0680	ribonucleoside-diphosphate reductase 1 alpha subunit	1.873	2.173	2.238	1.870
Biosynthesis of cofactors, prosthetic groups, and carriers					
HP0604	uroporphyrinogen decarboxylase	1.380	1.759	2.043	1.730

Table 2. Continued

Gene ID	Gene functionand classification[a]	Fold change[b]			
		15 min	30 min	60 min	120 min
Fatty acid and phospholipid metabolism					
HP0561	3-ketoacyl-acyl carrier protein reductase	1.614	2.014	2.482	2.423
HP0962	acyl carrier protein	2.036	2.437	2.319	3.092
Amino acid synthesis					
HP0098	threonine synthase	1.767	2.016	1.989	1.992
DNA metabolism					
HP1369	type III restriction enzyme M protein	2.010	2.491	2.190	1.839
Regulatory functions					
HP1365	response regulator	1.259	2.334	2.551	3.135
Two-component system					
HP0166	response regulator	1.464	1.980	2.093	2.016
HP0165	hypothetical protein	1.715	2.739	2.865	3.330
Hypothetical protein					
HP1432	histidine and glutamine-rich protein	1.881	1.538	2.012	1.855
HP0148	hypothetical protein	1.795	2.048	1.804	1.967
HP0085	hypothetical protein	1.597	1.624	2.754	2.874
HP0111	hypothetical protein	3.622	3.703	4.076	3.671
HP0168	hypothetical protein	1.748	1.706	2.236	3.309
HP0187	hypothetical protein	1.462	1.500	1.804	2.246
HP0188	hypothetical protein	2.011	3.078	2.243	3.435
HP0337	hypothetical protein	1.296	1.285	1.729	2.653
HP0350	hypothetical protein	1.641	2.531	2.788	2.913
HP0385	hypothetical protein	1.958	2.056	2.626	2.983
HP0386	hypothetical protein	1.853	3.261	2.298	2.524
HP0563	hypothetical protein	2.024	2.750	2.902	2.847
HP0565	hypothetical protein	2.098	2.648	2.889	2.529
HP0573	hypothetical protein	1.410	1.524	1.763	2.622
HP0635	hypothetical protein	1.715	1.828	2.138	1.943
HP0641	hypothetical protein	2.050	2.384	2.326	2.864
HP0678	hypothetical protein	2.131	2.400	2.171	1.816
HP0704	hypothetical protein	1.760	1.912	1.806	3.046
HP0719	hypothetical protein	1.907	2.262	2.391	2.532
HP0720	hypothetical protein	1.787	1.952	1.975	2.218
HP0762	hypothetical protein	2.072	2.398	2.430	2.906
HP0767	hypothetical protein	2.084	2.944	2.902	4.125
HP0918	hypothetical protein	1.529	2.061	2.454	2.429
HP0938	hypothetical protein	2.318	2.655	2.254	2.522
HP1124	hypothetical protein	1.658	1.753	2.123	2.018
HP0318	conserved hypothetical protein	2.335	2.377	2.542	2.582

[a] Gene ID and functional categories of *H. pylori* genome were defined according to http://www.tigr.org/tigr-scripts/CMR2/CMRGenomes.spl.
[b] Induction ratios between test sample and reference sample of of *H. pylori* at different time points after thansfer to 20°C are displayed.

essential roles in the modulation of host immune responses (Zhang *et al*., 2005). Interestingly, we found genes encoding outer membrane proteins were differentially expressed, either induced (HP1125, HP0227, HP0229) or repressed (HP0913, HP0079, HP0693), suggesting that the cell possibly alters their membrane composition or structure at the two different temperatures.

Motility and chemotaxis genes

Motility and chemotaxis can enable *H. pylori* to penetrate host tissue barriers and are essential *in vivo* (Figura *et al*., 2004). Among *H. pylori* chemotaxis genes, HP0103, HP1192 were up-regulated after transfer to 20°C, which indicates it can move toward substrates under low temperature conditions. Bacterial chemotaxis is a complex signal transduction system, which connects environmental stimuli to the direc-

Table 3. Genes showing repression upon temperature downshift in *H. pylori*

Gene ID	Gene functionand classification[a]	Fold change[b]			
		15 min	30 min	60 min	120 min
DNA metabolism					
HP0877	Holliday junction endodeoxyribonuclease	0.438	0.450	0.399	0.438
HP1063	glucose-inhibited division protein	0.651	0.676	0.704	0.353
HP0995	integrase/recombinase	0.440	0.371	0.393	0.252
HP1366	type IIS restriction enzyme R protein	0.468	0.330	0.453	0.0753
HP0054	adenine/cytosine DNA methyltransferase	0.618	0.072	0.324	0.490
HP0592	type III restriction enzyme R protein	0.521	0.352	0.499	0.317
HP0593	adenine specific DNA methyltransferase	0.682	0.530	0.603	0.334
jhp0414	type I restriction enzyme (specificity subunit)	0.509	0.411	0.467	0.420
jhp0918	DNA transfer protein	0.606	0.444	0.441	0.323
jhp0726	type I restriction enzyme (specificity subunit)	0.479	0.434	0.433	0.310
jhp0046	type II restriction enzyme	0.503	0.565	0.552	0.338
Cell envelope (Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides)					
HP1392	fibronectin/fibrinogen-binding protein	0.552	0.578	0.581	0.445
HP1429	polysialic acid capsule expression protein	0.193	0.152	0.130	0.136
HP0079	outer membrane protein	0.673	0.235	0.378	0.560
HP0913	outer membrane protein	0.568	0.433	0.516	0.514
HP0693	conserved hypothetical membrane protein	0.447	0.453	0.453	0.418
jhp0870	putative outer membrane protein	0.475	0.390	0.382	0.363
Cellular processes					
HP0017	*virB4* homolog	0.618	0.407	0.505	0.528
HP0042	trbI protein	0.555	0.381	0.487	0.479
HP0115	flagellin B	0.446	0.356	0.361	0.332
HP0870	flagellar hook	0.556	0.474	0.535	0.539
HP1452	thiophene and furan oxidizer	0.432	0.459	0.439	0.456
HP1431	16S rRNA (adenosine-N6,N6-) - dimethyltransferase	0.434	0.392	0.407	0.501
Protein synthesis (Translation factors)					
HP1298	translation initiation factor EF-1	0.488	0.632	0.575	0.685
Ribosomal proteins: synthesis and modification					
HP1301	ribosomal protein L15	0.368	0.449	0.488	0.489
HP1305	ribosomal protein S8	0.424	0.539	0.513	0.576
HP1307	ribosomal protein L5	0.382	0.421	0.436	0.440
Central intermediary metabolism					
HP0696	N-methylhydantoinase	0.624	0.496	0.486	0.469
Amino acid synthesis					
HP0096	phosphoglycerate dehydrogenase	0.508	0.334	0.279	0.265
HP0663	chorismate synthase	0.650	0.390	0.397	0.476
HP0695	hydantoin utilization protein A	0.590	0.447	0.454	0.391
HP1050	homoserine kinase	0.667	0.548	0.516	0.465
Energy metabolism (ferment)					
HP0691	3-oxoadipate coA-transferase subunit A	0.493	0.439	0.395	0.396
HP0692	3-oxoadipate coA-transferase subunit B	0.425	0.38	0.333	0.375
Transport and binding proteins					
HP0613	ABC transporter, ATP-binding protein	0.722	0.529	0.486	0.593
Protein fate-Protein and peptide secretion and trafficking					
HP1300	preprotein translocase subunit	0.351	0.458	0.460	0.442

Table 3. Continued

Gene ID	Gene functionand classification[a]	Fold change[b]			
		15 min	30 min	60 min	120 min
Transcription					
HP1198	DNA-directed RNA polymerase, beta subunit	0.461	0.444	0.439	0.239
Hypothetical protein					
HP1007	transposase-like protein, PS3IS, authentic frameshift	0.715	0.729	0.563	0.419
HP1000	PARA protein	0.573	0.490	0.479	0.305
HP0660	hypothetical protein	0.499	0.553	0.587	0.785
HP0052	hypothetical protein	0.398	0.106	0.221	0.362
HP0097	hypothetical protein	0.664	0.442	0.378	0.371
HP0114	hypothetical protein	0.471	0.400	0.402	0.419
HP0711	hypothetical protein	0.772	0.578	0.486	0.525
HP0985	hypothetical protein	0.675	0.558	0.593	0.445
HP1002	hypothetical protein	0.631	0.539	0.552	0.376
HP1003	hypothetical protein	0.681	0.603	0.644	0.449
HP0659	hypothetical protein	0.465	0.411	0.424	0.474
HP1077	hypothetical protein	0.684	0.503	0.415	0.488
HP1174	hypothetical protein	0.520	0.551	0.528	0.469
HP1215	hypothetical protein	0.741	0.763	0.705	0.481
HP1290	hypothetical protein	0.548	0.427	0.362	0.358
HP1430	conserved hypothetical ATP-binding protein	0.290	0.216	0.213	0.216
HP0258	conserved hypothetical membrane protein	0.474	0.506	0.530	0.539
HP0373	conserved hypothetical protein	0.574	0.487	0.431	0.421
HP1049	conserved hypothetical protein	0.623	0.556	0.462	0.512
HP1242	conserved hypothetical protein	0.772	0.596	0.506	0.492
HP1428	conserved hypothetical protein	0.178	0.154	0.116	0.141
HP0710	conserved hypothetical protein	0.435	0.351	0.341	0.325
jhp0921	hypothetical protein	0.692	0.558	0.603	0.441
jhp0937	hypothetical protein	0.534	0.446	0.466	0.381
jhp0949	hypothetical protein	0.653	0.545	0.559	0.430
jhp0755	hypothetical protein	0.668	0.554	0.646	0.434
jhp0960	hypothetical protein	0.634	0.579	0.492	0.403
jhp0165	hypothetical protein	0.457	0.372	0.373	0.285
jhp0927	hypothetical protein	0.682	0.568	0.613	0.469
jhp0935	hypothetical protein	0.504	0.568	0.465	0.343
jhp1160	hypothetical protein	0.809	0.665	0.626	0.451
jhp1463	hypothetical protein	0.334	0.278	0.316	0.199
jhp0926	hypothetical protein	0.729	0.648	0.597	0.446
jhp0933	hypothetical protein	0.554	0.485	0.478	0.348
jhp0923	hypothetical protein	0.742	0.589	0.556	0.394
jhp0940	hypothetical protein	0.637	0.545	0.554	0.478
jhp0820	putative lipopolysaccharide biosynthesis protein	0.550	0.457	0.433	0.406

[a] Gene ID and functional categories of *H. pylori* genome were defined according to http://www.tigr.org/tigr-scripts/CMR2/CMRGenomes.spl.
[b] Expression ratios of repressed genes between test sample and reference sample of of *H. pylori* at different time points after thansfer to 20°C are displayed.

tion of flagellar rotation and, thus, to the direction of movement. Chemotaxis plays multiple roles in the adaptation of a bacterium to its environment and contributes to its ability to colonize (McGee *et al*., 2005; Rajagopala *et al*., 2007). *H. pylori* mutants lacking the TlpB chemoreceptor had a mouse colonization defect (Croxen *et al*., 2006). Thus, the chemotaxis system of *H. pylori* may be another example of the remarkable ability of *H. pylori* to adapt to common physiological systems.

The flagellar system of *H. pylori* consists of more than 40, mostly unclustered genes. Flagella are essential for *H. pylori* to colonize its host and to orient itself in gradients essential for energy generation (Schreiber *et al*., 2004). Little is known about the regulatory mechanism of the expression of the flagellum genes or the flagellar assembly genes. In this study, HP0325 (flagellar basal-body L-ring protein), HP0684 (flagellar biosynthesis protein, authentic frameshift) were up-regulated, and HP0115 (flagellin B), HP0870 (flagellar hook) were down-regulated, which suggested that flagellar gene expression was subject to temperature regulation. Budde *et al*. (2006) found that chemotaxis and motility genes of *B. subtilis* appear to be repressed at low temperatures, suggesting that the ability of cells to actively swim is reduced or abolished when *B. subtilis* is cultivated at low temperature.

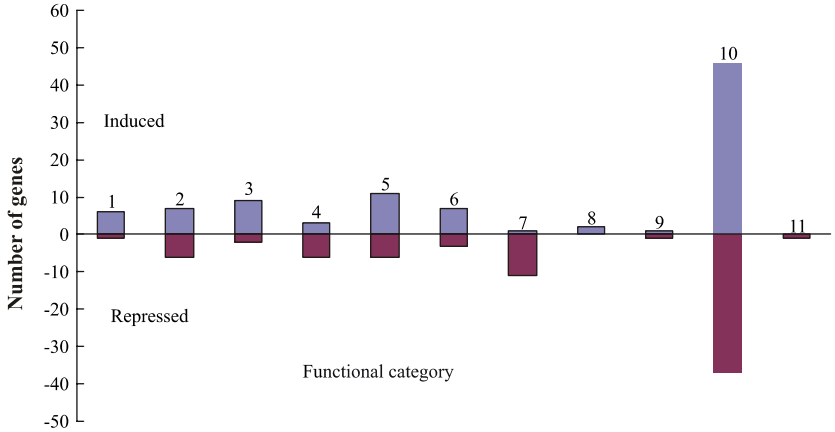

Fig. 2. Differentially expressed genes grouped by functional classification according to the *H. pylori* genome database from http://www.tigr.org. Blue bars refer to induced genes, and red bars refer to repressed genes. Columns: 1, protein fate; 2, cellular processes; 3, energy metabolism; 4, cell envelope; 5, substance metabolism (including protein, nucleotide, nucleic acid, cofactors and carriers; fatty acid, phospholipids biosynthesis and central intermediary metabolism); 6, ribosomal protein; 7, DNA metabolism; 8, regulatory functions; 9, transport and binding proteins; 10, hypothetical protein; 11, transcription.

Our study found that motility genes of *H. pylori* were differentially expressed, which indicates that temperature may influence motility of *H. pylori*.

The neutrophil activating protein gene (HP0243, *napA)* It was notable that among genes resistant to toxic damage, *H. pylori napA* was induced after transfer to 20°C. During the course of transmission, *H. pylori* is exposed to oxidative stress from the leakage of reactive oxygen species (Brisslert *et al*., 2005). *H. pylori napA* is a protective antigen and a major virulence factor that can mediate neutrophil adhesion to endothelial cells. *H. pylori napA* mutant does not survive as well as the wild-type strain upon exposure to oxidative stress conditions, suggesting that it plays a role in

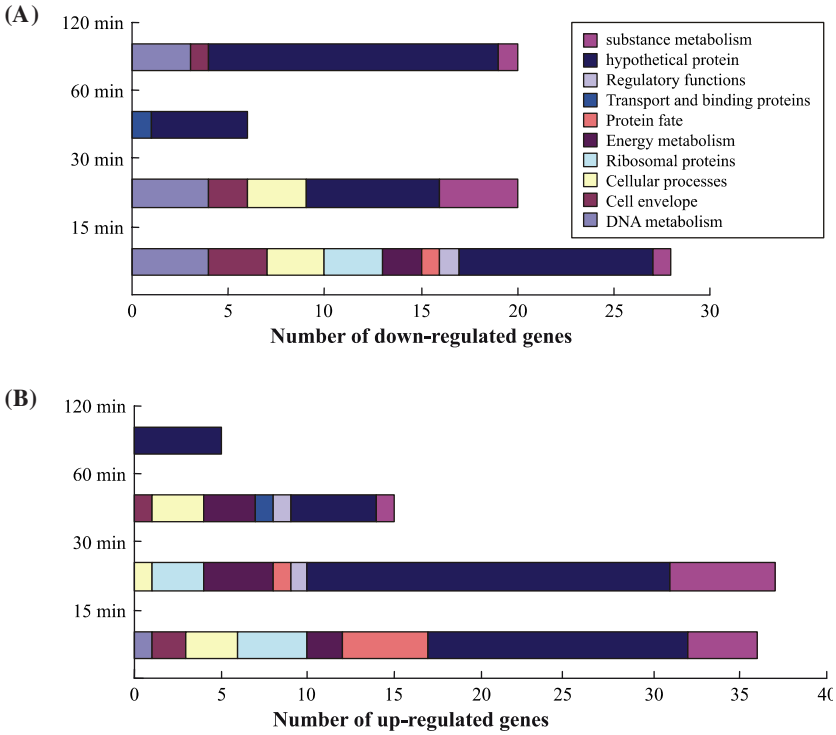

Fig. 3. Differentially expressed genes grouped by time points. X-axis represented the number of differentially expressed genes. Y-axis represented the four time points after transfer to 20°C. Different colors represented functional categories of *H. pylori* genome according to http://www.tigr.org/tigr-scripts/CMR2/CMRGenomes.spl.

protecting *H. pylori* from oxidative stress damage (Cooksley *et al*., 2003). Our study reveals that temperature downshifts up-regulate *H. pylori napA* expression at mid-log phase, which may increase survival of *H. pylori* in unfavorable environments. A cross-protection between the cold-shock response and oxidative-stress response might explain this increased resistance at low temperature (Garénaux *et al*., 2008).

Fatty acid and phospholipid metabolism genes

The acyl carrier protein (ACP) is a small, acidic protein and an essential component in the fatty acid biosynthesis pathway of bacteria. ACP also participates in other biosynthetic pathways involving the transfer of acyl groups, such as the biosynthesis of phospholipids (White *et al*., 2005; Liu *et al*., 2007). In our study, HP0962 (acyl carrier protein) and HP0561 (3-ketoacyl-acyl carrier protein reductase) were induced, indicating that temperature downshifts change the synthesis of *H. pylori* fatty acids. *Salmonella typhimurium* obtained at lower temperatures (10°C and 25°C) had an increase in the concentration of unsaturated fatty acids of the cytoplasmic membrane, whereas the concentration of saturated fatty acids remains almost constant, resulting in a higher membrane fluidity, which is linked to reduced thermal resistance (Alvarez-Ordóñez *et al*., 2008). Furthermore, membrane fatty acid composition and membrane fluidity can affect the bacterial thermotolerance (Sampathkumar *et al*., 2004). The up-regulation of *H. pylori* acyl carrier protein and ketoacyl-acyl carrier protein reductase genes suggests that cells adjust the lipid composition of the cytoplasmic membrane to sustain growth at low temperatures.

Two-component systems and other regulator genes

Two-component systems are signal transduction systems that allow regulation of cellular functions in response to changing environmental conditions. The *H. pylori* two-component system is composed of histidine kinase HP0165 and response regulator HP0166. Balanced synthesis of HP0166 is required in responding to environmental stimuli perceived by histidine kinase HP0165 (Dietz *et al*., 2002). In our study, HP0165 and HP0166 were up-regulated, which may be favorable for *H. pylori* growth and infection at low temperatures, but their exact mechanism in cold-shock response need to be elucidated.

Independent reports provide evidence that the general stress regulator plays a role in adaptation of bacteria to growth at low temperature (Brigulla *et al*., 2003). Thus, general stress proteins seem to be necessary for optimal growth. HP1496 (general stress protein) was induced after temperature transfer to 20°C, suggesting that the general stress response may promote the adaptation of *H. pylori* to growth at low temperature.

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References

Alvarez-Ordóñez, A., A. Fernández, M. López, R. Arenas, and A. Bernardo. 2008. Modifications in membrane fatty acid composition of *Salmonella typhimurium* in response to growth conditions and their effect on heat resistance. *Int. J. Food Microbiol.* 123, 212-219.

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

Beckering, C.L., L. Steil, M.H. Weber, U. Volker, and M.A. Marahiel. 2002. Genome-wide transcriptional analysis of the cold shock response in *Bacillus subtilis*. *J. Bacteriol.* 184, 6395-6402.

Bjorkholm, B.M., J.L. Guruge, J.D. Oh, A.J. Syder, N. Salama, K. Guillemin, S. Falkow, C. Nilsson, P.G. Falk, L. Engstrand, and J.I. Gordon. 2002. Colonization of germ-free transgenic mice with genotyped *Helicobacter pylori* strains from a case-control study of gastric cancer reveals a correlation between host responses and HsdS components of type I restriction-modification systems. *J. Biol. Chem.* 277, 34191-34197.

Brigulla, M., T. Hoffmann, A. Krisp, A. Völker, E. Bremer, and U. Völker. 2003. Chill induction of the SigB-dependent general stress response in *Bacillus subtilis* and its contribution to low-temperature adaptation. *J. Bacteriol*. 185, 4305-4314.

Brisslert, M., K. Enarsson, S. Lundin, A. Karlsson, J.G. Kusters, A.M. Svennerholm, S. Backert, and M. Quiding-Jarbrink. 2005. *Helicobacter pylori* induce neutrophil transendothelial migration: role of the bacterial HP-NAP. *FEMS Microbiol. Lett.* 249, 95-103.

Budde, I., L. Steil, C. Scharf, U. Volker, and E. Bremer. 2006. Adaptation of *Bacillus subtilis* to growth at low temperature: a combined transcriptomic and proteomic appraisal. *Microbiology* 152, 831-853.

Cooksley, C., P.J. Jenks, A. Green, A. Cockayne, R.P. Logan, and K.R. Hardie. 2003. NapA protects *Helicobacter pylori* from oxidative stress damage, and its production is influenced by the ferric uptake regulator. *J. Med. Microbiol.* 52, 461-469.

Crapoulet, N., P. Barbry, D. Raoult, and P. Renesto. 2006. Global Transcriptome analysis of *Tropheryma whipplei* in tesponse to temperature stresses. *J. Bacteriol.* 188, 5228-5239.

Croxen, M.A., G. Sisson, R. Melano, and P.S. Hoffman. 2006. The *Helicobacter pylori* chemotaxis receptor TlpB (HP0103) is required for pH taxis and for colonization of the gastric mucosa. *J. Bacteriol.* 188, 2656-2665.

De Jonge, R., Z. Durrani, S.G. Rijpkema, E.J. Kuipers, A.H. van Vliet, and J.G. Kusters. 2004. Role of the *Helicobacter pylori* outer-membrane proteins AlpA and AlpB in colonization of the guinea pig stomach. *J. Med. Microbiol.* 53, 375-379.

Derzelle, S., B. Hallet, K.P. Francis, T. Ferain, J. Delcour, and P. Hols. 2000. Changes in cspL, cspP, and cspC mRNA abundance as a function of cold shock and growth phase in *Lactobacillus plantarum. J. Bacteriol.* 182, 5105-5113.

Dietz, P., G. Gerlach, and D. Beier. 2002. Identification of target genes regulated by the two-component system HP166-HP165 of *Helicobacter pylori. J. Bacteriol.* 184, 350-362.

Figura, N., L. Trabalzini, R. Mini, G. Bernardini, A. Scaloni, F. Talamo, P. Lusini, E. Ferro, P. Martelli, and A. Santucci. 2004. Inactivation of *Helicobacter pylori cagA* gene affects motility. *Helicobacter* 9, 185-193.

Garénaux, A., F. Jugiau, F. Rama, R. de Jonge, M. Denis, M. Federighi, and M. Ritz. 2008. Survival of *Campylobacter jejuni* strains from different origins under oxidative stress conditions: effect of temperature. *Curr. Microbiol.* 56, 293-297.

Graumann, P., K. Schroder, R. Schmid, and M.A. Marahiel. 1996. Cold shock stress-induced proteins in *Bacillus subtilis*. *J. Bacteriol.* 178, 4611-4619.

Gualerzi, C.O., A.M. Giuliodori, and C.L. Pon. 2003. Transcriptional and post-transcriptional control of cold-shock genes. *J. Mol. Biol.* 331, 527-539.

Han, Y.H., W.Z. Liu, Y.Z. Shi, L.Q. Lu, S.D. Xiao, Q.H. Zhang, and G.P. Zhao. 2007. Comparative genomics profiling of clinical isolates of *Helicobacter pylori* in Chinese populations using DNA microarray. *J. Microbiol.* 45, 21-28.

Han, Y., D. Zhou, X. Pang, L. Zhang, Y. Song, Z. Tong, J. Bao, E. Dai, J. Wang, Z. Guo, J. Zhai, Z. Du, X. Wang, J. Wang, P. Huang, and R. Yang. 2005. DNA microarray analysis of the heat- and cold-shock stimulons in *Yersinia pestis*. *Microbes Infect.* 7, 335-348.

Hegarty, J.P., M.T. Dowd, and K.H. Baker. 1999. Occurrence of *Helicobacter pylori* in surface water in the United States. *J. Appl. Microbiol.* 87, 697-701.

Homuth, G., S. Domm, D. Kleiner, and W. Schumann. 2000. Transcriptional analysis of major heat shock genes of *Helicobacter pylori. J. Bacteriol.* 182, 4257-4263.

Jones, P., G.M. Cashel, G. Glaser, and F.C. Neidhardt. 1992. Function of a relaxed-like state following temperature downshifts in *Escherichia coli. J. Bacteriol.* 174, 3903-3914.

Lee, J.H., M.A. Heo, J.H. Seo, J.H. Kim, B.G. Kim, and S.G. Lee. 2008. Improving the growth rate of *Escherichia coli* DH5α at low temperature through engineering of GroEL/S chaperone system. *Biotechnol. Bioeng.* 99, 515-520.

Liu, W., L. Du, L. Zhang, J. Chen, X. Shen, and H. Jiang. 2007. *Helicobacter pylori* acyl carrier protein: expression, purification, and its interaction with beta-hydroxyacyl-ACP dehydratase. *Protein Expr. Purif.* 52, 74-81.

McGee, D.J., M.L. Langford, E.L. Watson, J.E. Carter, Y.T. Chen, and K.M. Ottemann. 2005. Colonization and inflammation deficiencies in *Mongolian gerbils* infected by *Helicobacter pylori* chemotaxis mutants. *Infect. Immun.* 73, 1820-1827.

Moreno, Y., M.A. Ferrus, J.L. Alonso, A. Jimenez, and J. Hernandez. 2003. Use of fluorescent *in situ* hybridization to evidence the presence of *Helicobacter pylori* in water. *Water Res.* 37, 2251-2256.

Phadtare, S. and M. Inouye. 2004. Genome-wide transcriptional analysis of the cold shock response in wild-type and coldsensitive, quadruple-csp-deletion strains of *Escherichia coli. J. Bacteriol.* 186, 7007-7014.

Rajagopala, S.V., B. Titz, J. Goll, J.R. Parrish, K. Wohlbold, M.T. McKevitt, T. Palzkill, H. Mori, R.L. Finley, Jr., and P. Uetz. 2007. The protein network of bacterial motility. *Mol. Syst. Biol.* 3, 128.

Salama, N., K. Guillemin, T.K. McDaniel, G. Sherlock, L. Tompkins, and S. Falkow. 2000. A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. *Proc. Natl. Acad. Sci. USA* 97, 14668-14673.

Sampathkumar, B., G.G. Khachatourians, and D.R. Korber. 2004. Treatment of *Salmonella enterica* Serovar Enteritidis with a sublethal concentration of trisodium phosphate or alkaline pH induces thermotolerance. *Appl. Environ. Microbiol.* 70, 4613-4620.

Schilling, C.H., M.W. Covert, I. Famili, G.M. Church, J.S. Edwards, and B.O. Palsson. 2002. Genome-scale metabolic model of *Helicobacter pylori* 26695. *J. Bacteriol.* 184, 4582-4593.

Schreiber, S., M. Konradt, C. Groll, P. Scheid, G. Hanauer, H.O. Werling, C. Josenhans, and S. Suerbaum. 2004. The spatial orientation of *Helicobacter pylori* in the gastric mucus. *Proc. Natl. Acad. Sci. USA* 101, 5024-5029.

Schumann, W., M. Hecker, and T. Msadek. 2002. Regulation and function of heat-inducible genes in *Bacillus subtilis*, p. 359-368. *In* A.L. Sonenshein, J.A. Hoch, and R. Losick (eds.), *Bacillus subtilis* and its closest relatives: from genes to cells. ASM Press, Washington, D.C., USA.

Smoot, L.M., J.C. Smoot, M.R. Graham, G.A. Somerville, D.E. Sturdevant, C.A. Migliaccio, G.L. Sylva, and J.M. Musser. 2001. Global differential gene expression in response to growth temperature alteration in group A *Streptococcus*. *Proc. Natl. Acad. Sci. USA* 98, 10416-10421.

Srikhanta, Y.N., T.L. Maguire, K.J. Stacey, S.M. Grimmond, and M.P. Jennings. 2005. The phasevarion: a genetic system controlling coordinated, random switching of expression of multiple genes. *Proc. Natl. Acad. Sci. USA* 102, 5547-5551.

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

Susin, M.F., R.L. Baldini, F. Gueiros-Filho, and S.L. Gomes. 2006. GroES/GroEL and DnaK/DnaJ have distinct roles in stress responses and during cell cycle progression in *Caulobacter crescentu. J. Bacteriol.* 188, 8044-8053.

Thompson, L.J., D.S. Merrell, B.A. Neilan, H. Mitchell, A. Lee, and S. Falkow. 2003. Gene expression profiling of *Helicobacter pylori* reveals a growth phase dependent switch in virulence gene expression. *Infect. Immun.* 71, 2643-2655.

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

White, S.W., J. Zheng, Y.M. Zhang, and C.O. Rock. 2005. The structural biology of type II fatty acid biosynthesis. *Annu. Rev. Biochem.* 74, 791-831.

Yoshimune, K., A. Galkin, L. Kulakova, T. Yoshimura, and N. Esaki. 2005. Cold-active DnaK of an antarctic psychrotroph *Shewanella* sp. Ac10 supporting the growth of dnaK-null mutant of *Escherichia coli* at cold temperatures. *Extremophiles* 9, 145-150.

Zhang, X.Y., Y. Yu, P. He, Y.X. Zhang, B.Y. Hu, Y. Yang, Y.X. Nie, X.G. Jiang, G.P. Zhao, and X.K. Guo. 2005. Expression and comparative analysis of genes encoding encoding outer membrane proteins LipL21, LipL32 and OmpL1 in *epidemic leptospires. Acta. Biochim. Biophys. Sin.* 37, 649-656.