Global Transcriptome Analysis of *Tropheryma whippelii* in Response to Temperature Stresses†

Nicolas Crapoulet,¹ Pascal Barbry,² Didier Raoult,¹ and Patricia Renesto^{1*}

Unité des Rickettsies, UMR 6020 CNRS, IFR48, Faculté de Médecine, 27, Boulevard Jean Moulin, Marseille, France,¹
and Institut de Pharmacologie Moléculaire et Cellulaire, UMR 6097 CNRS/UNSA, Sophia Antipolis, France²

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Tropheryma whippelii, the agent responsible for Whipple disease, is a poorly known pathogen suspected to have an environmental origin. The availability of the sequence of the 0.92-Mb genome of this organism made a global gene expression analysis in response to thermal stresses feasible, which resulted in unique transcription profiles. A few genes were differentially transcribed after 15 min of exposure at 43°C. The effects observed included up-regulation of the *dnaK* regulon, which is composed of six genes and is likely to be under control of two HspR-associated inverted repeats (HAIR motifs) found in the 5' region. Putative virulence factors, like the RibC and IspDF proteins, were also overexpressed. While it was not affected much by heat shock, the *T. whippelii* transcriptome was strongly modified following cold shock at 4°C. For the 149 genes that were differentially transcribed, eight regulons were identified, and one of them was composed of five genes exhibiting similarity with genes encoding ABC transporters. Up-regulation of these genes suggested that there was an increase in nutrient uptake when the bacterium was exposed to cold stress. As observed for other bacterial species, the major classes of differentially transcribed genes encode membrane proteins and enzymes involved in fatty acid biosynthesis, indicating that membrane modifications are critical. Paradoxically, the heat shock proteins GroEL2 and ClpP1 were up-regulated. Altogether, the data show that despite the lack of classical regulation pathways, *T. whippelii* exhibits an adaptive response to thermal stresses which is consistent with its specific environmental origin and could allow survival under cold conditions.

Tropheryma whippelii is an emerging human pathogen and is responsible for Whipple's disease (59). This disease is characterized by intestinal malabsorption leading to cachexia, but cardiac or central nervous system involvement, not always associated with digestive symptoms, may also be observed (55). Without treatment, its natural evolution is always fatal. Despite an antibiotic regimen, clinical relapses that take the form of cerebral involvement may be observed (31, 32).

T. whippelii is a gram-positive bacterium which for a long time was thought to be resistant to cultivation, but it was isolated in eukaryotic cells and propagated in culture at 37°C in 2000 (45). This has enabled further characterization of this pathogen, including genome sequencing (6, 44). With a 0.92-Mb genome, *T. whippelii*, which is located phylogenetically in the high-G+C-content gram-positive bacterial group between the genus *Cellulomonas* and the actinomycete clade (36), is a reduced-genome species belonging to the *Actinobacteria* with lower G+C contents. Like other reduced-genome bacteria, *T. whippelii* does not have several genes that regulate transcription and are classified in the K functional category in the Clusters of Orthologous Groups database. Based on the intracellular nature of these microorganisms, it was hypothesized that this deficiency was due to the rather stable environment inside host cells, which makes extensive gene regulation useless (34). To date, very little is known about the natural habitats of *T. whippelii* and the way that it infects hosts. Therefore, while *T. whippelii* does not contain most regula-

tory elements, this microorganism is thought to be an environmental agent (37) that is able to adapt to a wide variety of stress conditions.

Temperature change is the most common stress that all living organisms encounter in natural habitats. To overcome critical situations which could be generated by extreme temperatures, bacteria have evolved complex and specific mechanisms that are called cold shock and heat shock responses (43, 57). To deal with heat stress, bacteria overexpress heat shock proteins (HSP), including chaperones encoded by the *dnaK* and *groE* operons and ATP-dependent proteases (Clp and Lon). Chaperones prevent misfolding and aggregation of partially denatured proteins, while proteases degrade these proteins (21). Expression of HSP is regulated at the transcriptional level by various positive and negative mechanisms, depending on the bacterium. Positive control is provided by alternative sigma factors that target RNA polymerase to the heat shock gene promoter (22). In gram-positive bacteria, HSP are regulated mainly by less sophisticated mechanisms, called repressor mechanisms, which arose early in evolution (40). Such regulatory elements correspond to specific DNA sequences located in promoter regions, providing a simple and economical regulation pathway. An inverted repeat having a conserved consensus sequence (TTGGCGCTC-N₉-GAGTGCTAA) and termed CIRCE (controlling inverted repeat of chaperone expression) (63) has been found in several bacteria in association with the *groESL* or *dnaK* operon. Another DNA sequence, the HspR-associated inverted repeat (HAIR motif), was described as a regulon under the control of HspR (50). This regulon has been found mainly in high-G+C-content gram-positive bacteria, particularly actinomycetes (52). HSP are classified according to their regulation characteristics, which differ for each bacterial

* Corresponding author. Mailing address: Unité des Rickettsies, CNRS UMR6020, IFR48, Faculté de Médecine, 27, Boulevard Jean Moulin, 13385 Marseille, France. Phone: (33) 491 32 43 75. Fax: (33) 491 38 77 72. E-mail: patricia.renesto@medecine.univ-mrs.fr.

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species. In gram-positive bacteria, four regulatory classes have been defined. The class I genes include the genes encoding the classical chaperones DnaK, GroES, and GroEL that are negatively controlled by the *hrcA*-encoded protein which binds to the CIRCE sequence (26). The class II genes include genes encoding more than 40 stress proteins regulated by a σ^B factor. Class III gene expression, under negative control of the class III stress gene repressor (*ctrR*), recognizes a repeat sequence in the promoter. Class IV comprises other genes whose regulation is not known (9). Overlap between class I and class III HSP has been observed for streptococci (9).

The molecular basis of the cold shock response is also well documented. Bacteria respond to low temperatures by induction of a set of proteins that include the cold shock protein (CSP) family, which is autoregulated by an unusual long 5' untranslated region in the mRNA transcript (43). The high level of conservation of CSP in different bacterial lineages suggests that such proteins are very ancient. Moreover, the presence of cold shock domains in both eubacteria and eukaryotes indicates that cold shock domain-like proteins were present before the divergence of bacteria and eukarya/archaea (19). While this ancient family of small proteins is universally conserved among bacteria, a few exceptions have been observed in archaea (*Methanococcus jannaschii*, *Methanococcus thermoautotrophicum*, and *Archaeoglobus fulgidus*) and some bacteria, such as *Synechocystis* sp. strain PCC6803 and *Borrelia burgdorferi* (19). *T. whippelii* also lacks CSP (6, 44). It has been hypothesized that in cyanobacteria CSP are replaced by small cold-inducible proteins (Rbps) homologous to the RNA-binding domain found in eukaryotic RNA-binding proteins (48), but no *rpb*-like sequence was found in *T. whippelii*.

Here, the adaptive responses and the regulation mechanisms of *T. whippelii* exposed to various sudden temperature shifts were investigated by using a specific microarray. This work was the first attempt to analyze the transcriptome regulation of this pathogenic bacterium whose origin is unknown.

MATERIALS AND METHODS

***T. whippelii* whole-genome microarray construction.** A total of 1,608 PCR primers were designed in order to PCR amplify 804 annotated open reading frames (ORFs) representing about 99.5% of the *T. whippelii* genome (44). The remaining genes (0.5% of the genome) cannot be targeted because their sequences share repeated regions that impair the design of specific probes.

The following criteria were used to identify optimal forward and reverse primers for generating PCR products specific for each of the selected ORFs: (i) the primers were highly specific for the *T. whippelii* genome; (ii) the amplicons were roughly 260 bp long; (iii) the amplicons were highly specific for the corresponding genes; (iv) each primer contained 20 to 25 bases, and the annealing temperature of the primers was ~60°C; and (v) each primer contained an additional sequence in the 5' area specific for forward and reverse primers. The universal sequences added were used for a second round of amplification.

ORF-specific fragments were amplified using *T. whippelii* DNA as the template under following conditions: 40 cycles of 20 s of denaturation at 95°C, 30 s of annealing at 60°C, and 30 s of extension at 72°C, with an initial 5 min of denaturation at 95°C and final extension at 72°C for 5 min. Following amplification, PCR products were visualized by ethidium bromide staining after migration in a 2% agarose gel. Purified amplicons (QIAquick 96-well purification kit; QIAGEN, Courtaboeuf, France) were sequenced (3100 genetic analyzer; Applied Biosystems, Courtaboeuf, France) to check the identity of amplified samples and were processed by performing a second PCR with universal primers.

Amplified DNA fragments resuspended in a 50% dimethyl sulfoxide solution were printed in quadruplicate on Nexterion Slide A+ (Schott AG, Mainz, Germany) by using an SDDC-2 (Bio-Rad, Marnes-la-Coquette, France). A set of 23 artificial genes that serve as analytical controls to validate, filter, and normalize

microarray data contained in a Universal ScoreCard kit (Amersham Biosciences, Orsay, France) was also printed on glass slides. The spotted slides were cross-linked by using a UV Stratalinker (Stratagene, La Jolla, CA) with a total energy of 250 mJ and then were stored in a dry environment at room temperature.

Strain, medium, and growth conditions. All experiments were performed with mid-log cultures of *T. whippelii* strain Twist (35) grown at 37°C in Dulbecco modified Eagle medium/F12 medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal calf serum, 1% L-glutamine, and 1% human nonessential amino acids (Invitrogen). Bacterial growth was monitored by flow cytometry counting using a Microcyte portable flow cytometer (Optoflow AS, Oslo, Norway) and by quantitative PCR as previously described (15, 46).

RNA purification. For the heat shock experiments, flasks containing bacteria were transferred into a water bath maintained at 43°C for the following times: 15, 30, and 60 min. For cold shock assays, a bacterial suspension was incubated at either 4°C or 28°C for 1 or 6 h. For all experimental conditions tested, the basal level of transcripts was estimated by using RNA extracted from different culture flasks collected separately before incubation under the temperature stress conditions. Considering both the culture phase and the low growth rate of this bacterium (28 h) (46), we assumed that the effects observed were due to the thermal stress. RNA was extracted by brief sonication of bacteria in 1 ml of Trizol reagent (Invitrogen Life Technologies) as described previously (11). Purified RNA samples resuspended in diethyl pyrocarbonate-treated water were treated with RNase-free DNase Set (QIAGEN) and cleaned on a RNeasy column (QIAGEN) to remove DNA contamination. The amount and quality of each RNA sample were checked by automated capillary gel electrophoresis using a Bioanalyzer 2100 with RNA Nano LabChips (Agilent, Palo Alto, CA). Four hundred milliliters of a *T. whippelii* axenic culture was required to obtain 10 µg of purified RNA.

RNA labeling. Fluorescently labeled cDNA was prepared using a CyScribe first-strand cDNA labeling kit (Amersham Biosciences). Briefly, 10 µg of total RNA supplemented with control RNAs (Universal ScoreCard kit; Amersham Biosciences) was annealed with random nonamer primers. Total RNA was directly reverse transcribed and labeled by incorporation of Cy3-dCTP or Cy5-dCTP (Amersham Biosciences) using CyScript reverse transcriptase. The remaining RNA was then degraded by alkaline hydrolysis treatment, and after neutralization, labeled first-strand cDNA was purified with a CyScribe GFX purification kit (Amersham Biosciences). Before hybridization, the levels of Cy3 and Cy5 incorporation were quantified by measuring the absorbance at 550 and 650 nm, respectively. Hybridizations were performed with incorporation levels ranging from 80 to 200 pmol of fluorochromes per µg of cDNA.

Hybridization and washing. Microarrays that were washed in 0.1% sodium dodecyl sulfate (SDS) (1 min) and rinsed with H₂O (1 min) were boiled for 3 min in H₂O in order to denature spotted double-strand DNA. Following prehybridization at 42°C for 45 min in 5× SSC–0.1% SDS–0.1% bovine serum albumin, the microarrays were rinsed in H₂O (1 min) and dried with compressed nitrogen (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Hybridization was then carried out using two samples of cDNA (10 µg each) that were labeled with Cy3- or Cy5-dCTP, pooled, evaporated (SpeedVac concentrator), and resuspended in 6 µl of nuclease-free H₂O. The mixture was heated at 95°C for at least 2 min and then cooled on ice for 30 s before addition of 7.5 µl of microarray hybridization buffer (supplied with the CyScribe kit) and 15 µl of 100% (vol/vol) formamide and applied to a microarray slide with a glass coverslip (24 by 60 mm). Following 18 h of hybridization at 42°C, the microarrays were washed in 2× SSC–0.2% SDS for 10 min, in 2× SSC, and then in 0.2× SSC. Finally, the microarrays were dried with compressed nitrogen and scanned at a resolution of 10 µm by using a ScanArray Express (Perkin-Elmer, Boston, MA).

Analysis of microarray data. All microarray results have been deposited in the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) (4) under GEO Series accession number GSE3693. Signal intensity and local background measurements were obtained for each spot by using the ScanArray Express microarray analysis system software (version 2.1.8; Perkin-Elmer). The data filtering and normalization results were then processed with the Microsoft Excel software. Spots with median background-corrected signal intensities in both channels that were less than twice the median background intensity were not included in further analyses. Data normalization was performed for the remaining spots by using total intensity normalization methods. The normalized test signal/reference signal log ratio for each spot was recorded. The data were processed by using the TMEV software (www.tigr.org/software/TM4/) (47). An analysis of variance test was applied to the data, and genes with a *P* value of <0.02 were considered to have significant differential expression. Significant changes in gene expression were identified with significance analysis microarrays (56) using two-class paired data and a 1.5-fold cutoff. All experiments were conducted three times, including dye swapping, which yielded 12 measurements per gene (representing four technical

FIG. 1. Relative normalized fluorescence intensities of DNA microarrays. (A) Comparison of cDNAs derived from the same culture of bacteria grown at 37°C. (B) Comparison of cDNAs derived from two independent cultures of bacteria grown at 37°C and at 4°C. (C) Comparison of cDNAs derived from two independent cultures of bacteria grown at 37°C and at 28°C. (D) Comparison of cDNAs derived from two independent cultures of bacteria grown at 37°C and at 43°C. The upper and lower dashed lines indicate 1.5-fold changes in the signal intensities.

and three biological replicates). Gene expression was determined by determining the mean of the 12 values obtained.

Real-time RT-PCR. Reverse transcription (RT) from 2 µg of total RNA with 1 µg of random hexamer primers was carried out to generate cDNA. A real-time PCR was then performed for each cDNA preparation (1:20 dilution), using the LightCycler system (Roche) together with the SYBR Green master mixture. Eight genes exhibiting either up- or down-regulation (as shown by microarray data) were selected, as were invariant targets (namely, *leuS*, *mgt*, and TWT639). The levels of expression of these three genes, which were not sensitive to temperature changes, were used for normalization of real-time RT-PCR values. For each primer pair, a standard curve was constructed with genomic DNA of *T. whipplei* as the template. The relative expression ratios of target genes were calculated using the Pfaffl model (42). This model incorporates the amplification efficiencies of the target and reference (normalization) genes to correct for differences between the two assays.

Identification of potential DNA binding sites. Inverted-repeat elements, namely CIRCE (63) and HAIR (18) motifs, were searched using the Genome2D software (3, 63) with the whole *T. whipplei* genome (44).

RESULTS

Validation of *T. whipplei* microarray. In preliminary experiments, the reliability of the microarray data was assessed by cohybridization of two cDNA samples prepared from the same total RNA extract and labeled with either the Cy3 or Cy5 dye. The pattern of hybridization showed a linear correlation with no more than a 1.5-fold change in the relative expression level

(Fig. 1A), a cutoff value that was used in several previous studies (27, 51, 54). Thus, we focused on the genes that exhibited an expression ratio greater than this value. The transcription profiles of *T. whipplei* grown at 37°C and then subjected either to heat shock (43°C) or to cold shock (28°C and 4°C) are shown in Fig. 1 and Table 1. Microarray data were subsequently confirmed by real-time RT-PCR performed for 11 targets sharing various transcriptional patterns (Fig. 2 and Table 2). Thus, linear regression carried out with the plotted log ratio values obtained with each method resulted in a correlation coefficient (r) of 0.958 and a slope of 1.0276.

***T. whipplei* genes regulated upon heat shock.** The *T. whipplei* transcriptome profile was marginally affected by exposure of this microorganism to heat (Fig. 1D). Only 19 genes were differentially expressed ($P < 0.02$), and they were mainly up-regulated (Table 1). The data obtained were also expressed in histograms showing the percentages of differentially expressed genes classified according to their functional categories (Fig. 3). Genes encoding proteins involved in protein fate, as well as in protein folding and stabilization, such as *grpE*, *hspR*, *dnaK*, *clpB*, and *cbpA*, were specifically up-regulated. As shown in Table 1, for this category both the percentage and the intensity of changes (up to 15-fold increases) were higher than the percentage intensity of changes for other genes. The heat induction of *dnaK* was maximal after 15 min of exposure to 43°C and was not accompanied by up-regulation of *groEL* (Fig. 4). Two genes were down-regulated in response to heat shock. One of these genes is involved in central intermediary metabolism (*pho2*, encoding sugar phosphatase), and the other is involved in transport (*livH*, encoding an ABC transporter). Both of these genes were up-regulated by the same order of magnitude following cold shock at 4°C. Six other genes (*ribC*, *pho2*, *clpB*, *cbpA*, *livH*, and TWT751) were antagonistically regulated at 43°C and 4°C (Table 1; see Fig. S1 in the supplemental material).

Genetic organization and transcriptional mapping of the *dnaK* operon. Microarray data obtained after exposure of *T. whipplei* to heat shock showed that *grpE*, *hspR*, *dnaK*, *clpB*, and *cbpA* were cotranscribed along with TWT745, which encodes a protein whose function is not known. These genes are located at position 855189 through position 861728 in the *T. whipplei* Twist genome and probably constitute the *dnaK* operon. No CIRCE sequence was found upstream of *dnaK*. This inverted-repeat chaperone expression operator is controlled by the protein encoded by *hrcA*, which is also not present in the *T. whipplei* genome. The presence of the *hspR* gene led us to look for the HspR consensus binding sequence 5'-CTTGAGT-N₇-ACTCAAG-3' (18) in the 5' region of this heat shock operon. This resulted in identification of HAIR motifs upstream of the *dnaK* gene (Fig. 5). The sequences of these motifs, designated TW_HAIR1 and TW_HAIR2, were 5'-CATGAGTCGATATGACTCAAT-3' and 5'-CTTGAGTCAT TACATGTCAAG-3', respectively. A very similar profile was found in the *T. whipplei* TW08/27 genome (not shown). These motifs were found only in the *dnaK* operon and were not found in other regions of the *T. whipplei* genome.

***T. whipplei* genes regulated upon cold shock.** Following a temperature shift from 37°C to either 28°C or 4°C for 1 h, the *T. whipplei* transcriptome was unchanged (not shown). When the incubation time was increased to 6 h, 17 genes were differentially expressed at 28°C (9 genes were up-regulated, and 8 genes were down-regulated). Following 6 h of incubation at 4°C, 149 genes

TABLE 1. Genes with significant differences ($P < 0.02$ and ≥ 1.5 -fold change) between expression at 37°C and expression at 4°C, 28°C, and 43°C

Gene ID	Gene name	Fold changes at:			Product(s)	Proven or predicted function
		4°C	28°C	43°C		
Amino acid biosynthesis						
TWT567	<i>aroA</i>			1.5 ^a	3-Phosphoshikimate 1-carboxyvinyltransferase (EC 2.5.1.19)	Aromatic amino acid family
TWT162	<i>metE</i>	1.7			5-Methyltetrahydropterpyltryglutamate-homocysteine methyltransferase (EC 2.1.1.14)	Aspartate family
TWT635	<i>glyA^b</i>	1.9	1.5		Serine hydroxymethyltransferase (EC 2.1.2.1)	Serine family
TWT370	<i>aroB</i>	−2.5	−1.7		3-Dehydroquinate synthase (EC 4.2.3.4)	Aromatic amino acid family
Biosynthesis of cofactors prosthetic groups, and carriers						
TWT211	<i>bioY</i>	2			Biotin synthesis BioY protein	Biotin
TWT635	<i>glyA^b</i>	1.8	1.5		Serine hydroxymethyltransferase (EC 2.1.2.1)	Folic acid
TWT634	<i>folD</i>	1.6			Methylenetetrahydrofolate dehydrogenase (NADP ⁺)/methenyltetrahydrofolate cyclohydrolase (EC 1.5.1.5, 3.5.4.9)	Folic acid
TWT587	<i>folE</i>			3.1	GTP cyclohydrolase 1 (EC 3.5.4.16)	Folic acid
TWT068	<i>menD</i>	2			2-Oxoglutarate decarboxylase/2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase (EC 2.5.1.64)	Menaquinone and ubiquinone
TWT348	<i>ispDF</i>			3.8	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase/2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12)	Other
TWT103		1.6			Inorganic polyphosphate/ATP-NAD kinase (EC 2.7.1.23)	Pyridine nucleotides
TWT264		1.9			Pyridoxine biosynthesis enzyme-like protein	Pyridoxine
TWT476	<i>folC</i>	−1.7			Folypolyglutamate synthase (EC 6.3.2.17, 6.3.2.12)	Folic acid
TWT732	<i>hemC</i>	−1.7			Porphobilinogen deaminase (EC 2.5.1.61)	Heme, porphyrin, and cobalamin
TWT623		−2			Nicotinate phosphoribosyltransferase, putative	Pyridine nucleotides
TWT688	<i>ribC</i>	−2		12	Riboflavin synthase alpha chain (EC 2.5.1.9)	Riboflavin, flavin mononucleotide, and flavin adenine dinucleotide
Cell envelope						
TWT226	<i>murD</i>	3	1.6		UDP-N-acetylmuramoylalanine-D-glutamate ligase (EC 6.3.2.9)	Biosynthesis and degradation of murein sacculus and peptidoglycan
TWT031	<i>rmlC</i>	1.5			dTDP-4-dehydrorhamnose 3,5-epimerase (EC 5.1.3.13)	Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides
TWT117		2.4			Membrane protein, putative	Other
TWT673		1.8			Lipoprotein, putative	Other
TWT739		1.5			Lipoprotein, putative	Other
TWT482				3.3	Membrane protein, putative	Other
TWT505	<i>ddlA</i>	−1.7			D-Alanine-D-alanine ligase (EC 6.3.2.4)	Biosynthesis and degradation of murein sacculus and peptidoglycan
TWT160	— ^b	−1.7			Glycosyltransferase domain containing protein	Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides
TWT038		−2			Glycosyltransferase	Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides
TWT311		−1.7			Membrane protein, putative	Other
TWT583		−1.7			Putative ABC-2-type transport system permease protein	Other
TWT671		−1.7			Membrane protein, putative	Other
TWT569	— ^b	−2			Possible integral membrane protein	Other
Cellular processes						
TWT775	<i>ftsW</i>	2.6			Cell division protein FtsW	Cell division
TWT200	<i>ftsX^b</i>	1.6			Cell division protein FtsX	Cell division
TWT283	— ^b	1.5			Predicted metal-dependent hydrolase	Toxin production and resistance

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TABLE 1—Continued

Gene ID	Gene name	Fold changes at:			Product(s)	Proven or predicted function
		4°C	28°C	43°C		
TWT662	— ^b	−1.5			Iron ABC transporter substrate-binding protein	Cell adhesion/pathogenesis
TWT802	<i>parA1</i>	−1.7			Chromosome partitioning protein ParA	Cell division
TWT569	— ^b	−2			Possible integral membrane protein	Detoxification/toxin production and resistance
TWT384	<i>comEA</i>	−1.7			ComE operon protein 1-like protein	DNA transformation
Central intermediary metabolism						
TWT742	<i>pho2</i>	1.7		−1.7	Sugar phosphatase	Amino sugars
TWT283	— ^b	1.5			Predicted metal-dependent hydrolase	Other
TWT633	<i>gabT</i>	3.6	1.9		4-Aminobutyrate aminotransferase (EC 2.6.1.19)	Other
DNA metabolism						
TWT643	<i>xseA</i>	2.1			Exodeoxyribonuclease VII large subunit (EC 3.1.11.6)	Degradation of DNA
TWT182	<i>trcF</i>	1.9	1.6		Transcription-repair coupling factor	DNA replication, recombination, and repair
TWT006	<i>gyrA1</i>	1.7			DNA gyrase subunit A (EC 5.99.1.3)	DNA replication, recombination, and repair
TWT292	<i>polA</i>	1.7			DNA polymerase I (EC 2.7.7.7)	DNA replication, recombination, and repair
TWT325		−2			DNase (EC 3.1.21.-)	Degradation of DNA
TWT465	<i>ogt</i>	−1.5			Methylated-DNA-protein-cysteine methyltransferase (EC 2.1.1.63)	DNA replication, recombination, and repair
TWT611	<i>recA</i>	−1.6			Recombinase A	DNA replication, recombination, and repair
TWT569	— ^b	−2			Possible integral membrane protein	DNA replication, recombination, and repair
TWT001	<i>dnaA</i>	−2.6			Chromosomal replication initiator protein	DNA replication, recombination, and repair
Energy metabolism						
TWT769	<i>pdhB</i>	1.5			Pyruvate dehydrogenase E1 component beta subunit (EC 1.2.4.1)	Amino acids and amines
TWT424	<i>atpD</i>	1.7			ATP synthase beta chain (EC 3.6.3.14)	ATP-proton motive force interconversion
TWT283	— ^b	1.5			Predicted metal-dependent hydrolase	Biosynthesis and degradation of polysaccharides
TWT248	<i>qcrA</i>	1.6			Ubiquinol-cytochrome <i>c</i> reductase iron-sulfur subunit	Electron transport
TWT757	<i>ccsA</i>	1.6			Cytochrome <i>c</i> -type biogenesis protein	Electron transport
TWT213	<i>glkA</i>	2.2	1.6		Glucokinase (EC 2.7.1.2)	Glycolysis/gluconeogenesis
TWT630	<i>pca</i>	2			Pyruvate carboxylase (EC 6.4.1.1)	Glycolysis/gluconeogenesis
TWT755		1.8			Phosphoglycerate mutase	Glycolysis/gluconeogenesis
TWT783	<i>eno</i>	1.6			Enolase (EC 4.2.1.11)	Glycolysis/gluconeogenesis
TWT655	<i>gpm</i>	1.5			Phosphoglycerate mutase (EC 5.4.2.1)	Glycolysis/gluconeogenesis
TWT397	<i>rpl</i>	1.5			Ribose-5-phosphate isomerase B (EC 5.3.1.6)	Pentose phosphate pathway
TWT429	<i>atpE</i>	−2			ATP synthase C chain (EC 3.6.3.14)	ATP-proton motive force interconversion
TWT244	<i>claD</i>	−1.6			Cytochrome <i>c</i> oxidase subunit I (EC 1.9.3.1)	Electron transport
Fatty acid and phospholipid metabolism						
TWT020	<i>bccA</i>	2.2			Biotin carboxylase (EC 6.3.4.14)	Biosynthesis
TWT400		1.6			Predicted thioesterase	Biosynthesis/degradation
TWT254	<i>tabD</i>	1.5			Malonyl coenzyme A-acyl carrier protein transacylase (EC 2.3.1.39)	Biosynthesis
TWT283	— ^b	1.5			Predicted metal-dependent hydrotase	Degradation
TWT251	<i>kosA</i>	−1.7			3-Oxoacyl-(acyl carrier protein) synthase (EC 2.3.1.41)	Biosynthesis
TWT445	<i>cdsA</i>	−1.7			Phosphalidate cytidyltransferase (EC 2.7.7.41)	Biosynthesis
Protein fate						
TWT481	<i>clpP1</i>	2.6			ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)	Degradation of proteins, peptides, and glycopeptides

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TABLE 1—Continued

Gene ID	Gene name	Fold changes at:			Product(s)	Proven or predicted function
		4°C	28°C	43°C		
TWT450		1.6			M23/M37 peptidase domain protein protein	Degradation of proteins, peptides, and glycopeptides
TWT625	<i>htrA</i>	1.5			Putative serine protease (EC 3.4.21.-)	Degradation of proteins, peptides, and glycopeptides
TWT271	<i>secD</i>	1.9			Preprotein translocase SecD subunit	Protein and peptide secretion and trafficking
TWT556	<i>ftsY</i>	1.8			Cell division protein FtsY	Protein and peptide secretion and trafficking
TWT441	<i>groEL2</i>	2.6			60-kDa chaperonin 2	Protein folding and stabilization
TWT749	<i>grpE</i>			6.3	HSP-70 cofactor GrpE	Protein folding and stabilization
TWT747	<i>hspR^b</i>			2.8	Heat shock regulator	Protein folding and stabilization
TWT750	<i>dnaK</i>			10.3	Chaperone protein	Protein folding and stabilization
TWT746	<i>clpB</i>	−1.5		1.8	ATP-dependent protease ATP-binding subunit	Protein folding and stabilization
TWT748	<i>cbpA</i>	−1.7	−1.7	15.2	Curved DNA-binding protein	Protein folding and stabilization
TWT160	— ^b	−1.7			Glycosyltransferase domain containing protein	Protein modification and repair
TWT522	<i>pepQ</i>	−2			Peptidase (EC 3.4.11.9)	Degradation of proteins, peptides, and glycopeptides
TWT569	— ^b	−2			Possible integral membrane protein	Degradation of proteins, peptides, and glycopeptides
Protein synthesis						
TWT651	<i>pth</i>	2.2			Peptidyl-tRNA hydrolase (EC 3.1.1.29)	Other
TWT113	<i>rpmB</i>	2.3			50S ribosomal protein L28	Ribosomal proteins: synthesis and modification
TWT166	<i>rplT</i>	2			50S ribosomal protein L20	Ribosomal proteins: synthesis and modification
TWT115	<i>rpsN</i>	1.6			30S ribosomal protein S14	Ribosomal proteins: synthesis and modification
TWT547	<i>rplP</i>	1.6			50S ribosomal protein L16	Ribosomal proteins: synthesis and modification
TWT120	<i>tuf</i>	2.1			Elongation factor EF-Tu (EC 3.6.5.3)	Translation factors
TWT164	<i>infC</i>			2.7	Translation initiation factor IF-3	Translation factors
TWT203	<i>ksgA</i>	1.7			Dimethyladenosine transferase (EC 2.1.1.-)	tRNA and rRNA base modification
TWT529	<i>rpsK</i>	−1.7			30S ribosomal protein S11	Ribosomal proteins: synthesis and modification
TWT677	<i>rpsL</i>	−2			3S ribosomal protein S12	Ribosomal proteins: synthesis and modification
TWT714	<i>rplK</i>	−2.5			5S ribosomal protein L11	Ribosomal proteins: synthesis and modification
TWT675	<i>fusA</i>	−2			Elongation factor EF-G (EC 3.6.5.3)	Translation factors
TWT087	<i>truB</i>	−1.7			tRNA pseudouridine synthase B (EC 4.2.1.70)	tRNA and rRNA base modification
Purines, pyrimidines, nucleotides, and nucleotides						
TWT635	<i>glyA^b</i>	1.9	1.5		Serine hydroxymethyltransferase (EC 2.1.2.1)	Purine ribonucleotide biosynthesis
TWT724	<i>purM</i>	1.8			Phosphoribosylformylglycinamide cyclo-ligase (EC 6.3.3.1)	Purine ribonucleotide biosynthesis
TWT109	<i>purC</i>	1.6			Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6)	Purine ribonucleotide biosynthesis
TWT723	<i>purF</i>	1.6			Amidophosphoribosyltransferase (EC 2.4.2.14)	Purine ribonucleotide biosynthesis
TWT008				1.7	Thiamine pyrophosphokinase	Purine ribonucleotide biosynthesis
TWT196	<i>pyrB</i>	1.6			Aspartate carbamoyltransferase (EC 2.1.3.2)	Pyrimidine ribonucleotide biosynthesis
TWT743	<i>umpA</i>	1.5			Orotate phosphoribosyltransferase (EC 2.4.2.10)	Pyrimidine ribonucleotide biosynthesis
TWT683	<i>nrdA</i>	−1.5			Ribonucleotide reductase alpha chain (EC 17.4.1)	2'-Deoxyribonucleotide metabolism
TWT187	<i>prsA</i>	−2			Ribosephosphate pyrophosphokinase (EC 2.7.6.1)	Purine ribonucleotide biosynthesis

Continued on following page

TABLE 1—Continued

Gene ID	Gene name	Fold changes at:			Product(s)	Proven or predicted function
		4°C	28°C	43°C		
TWT100	<i>pyrG</i>	−1.7			CTP synthase (EC 6.3.4.2)	Pyrimidine ribonucleotide biosynthesis
TWT018	<i>deoD</i>	−1.7			Purine nucleoside phosphorylase (EC 2.4.2.1)	Salvage of nucleosides and nucleotides
Regulatory functions						
TWT747	<i>hspR^b</i>			2.6	Heat shock regulator	DNA interactions
TWT129	<i>comFC</i>	1.6			ComF operon protein 3	Other
TWT216	<i>pknB</i>	−1.7			Serine/threonine protein kinase (EC 2.7.1.37)	Protein interactions
Transcription						
TWT562	<i>rnc</i>	1.8			RNase III (EC 3.1.26.3)	RNA processing
TWT800	<i>pcnA</i>	1.6			Poly(A) polymerase (EC 2.7.7.19)	RNA processing
TWT349	<i>carD</i>	3.4			Transcriptional regulator	Transcription factors
TWT622	<i>rph</i>	−2			RNase PH (EC 2.7.7.56)	Degradation of RNA
TWT474	<i>mg</i>	−2.5	−1.7		RNase G (EC 3.1.4.-)	Degradation of RNA
TWT528	<i>rpoA</i>	−1.5			DNA-directed RNA polymerase alpha chain (EC 2.7.7.6)	DNA-dependent RNA polymerase
TWT071	<i>rpoB</i>	−1.7			DNA-directed RNA polymerase beta chain (EC 2.7.7.6)	Transcription factors
Transcription and binding proteins						
TWT561		2			Branched-chain amino acid ABC transporter substrate-binding protein	Amino acids, peptides, and amines
TWT560	<i>llvH</i>	1.7	−1.7		Branched-chain amino acid ABC transporter permease protein	Amino acids, peptides, and amines
TWT059	<i>fepG</i>	1.5	1.5		Iron ABC transporter permease protein	Cations and iron-carrying compounds
TWT663				4.9	Iron ABC transporter ATP-binding protein	Cations and iron-carrying compounds
TWT200	<i>ftsX^b</i>	1.6			Cell division protein FtsX	Unknown substrate
TWT408		1.5			ABC transporter ATP-binding protein	Unknown substrate
TWT137	<i>dppA2</i>	−2			Dipeptide ABC transporter substrate-binding protein	Amino acids, peptides, and amines
TWT354	<i>pstC</i>	−1.7	−1.7		Phosphate transport system permease protein	Anions
TWT355	<i>phoS</i>	−3.3	−2		Phosphate transport system substrate-binding protein	Anions
TWT662	— ^b	−1.6			Iron ABC transporter substrate-binding protein	Cations and iron-carrying compounds
TWT060	<i>jepD</i>	−1.7			Iron ABC transporter permease protein	Cations and iron-carrying compounds
TWT057		−1.7			Iron ABC transporter substrate-binding protein	Unknown substrate
Unknown function						
TWT432	<i>rfc</i>	1.6			Undecaprenyl-phosphate alpha-N-acetylglucosaminyltransferase (EC 2.4.1.-)	Enzymes of unknown specificity
TWT220	<i>mraW</i>	1.6			S-Adenosyl-methyltransferase (EC 2.1.1.-)	Enzymes of unknown specificity
TWT015		3.3	1.7		WiSP family protein	
TWT212		2.2			WiSP family protein	
TWT232		2.2			Unknown	
TWT768		2.1	2.1		Unknown	
TWT470		2.1			Unknown	
TWT773		2.1			Unknown	
TWT398		1.8	1.6		Unknown	
TWT779		1.8			Unknown	
TWT219		1.7			MraZ protein	
TWT233		1.7			Unknown	
TWT278	<i>lepA</i>	1.6			GTP-binding protein LepA	
TWT504		1.6			Unknown	
TWT740		1.6			Unknown	
TWT741		1.6			Unknown	
TWT767		1.6			Unknown	
TWT764		1.6			WiSP family protein	
TWT406	<i>om</i>	1.5			Oligoribonuclease (EC 3.1.-.-)	
TWT174		1.5			Unknown	

Continued on following page

TABLE 1—Continued

Gene ID	Gene name	Fold changes at:			Product(s)	Proven or predicted function
		4°C	28°C	43°C		
TWT257	comM	1.5			Unknown	
TWT274		1.6			Unknown	
TWT319		1.5			Putative FKBP-type peptidyl-prolyl <i>cis-trans</i> isomerase	
TWT119		1.5			Unknown	
TWT454				5.4	Competence protein ComM	
TWT745				2.8	Unknown	
TWT652				1.6	Unknown	
TWT751		−1.7		1.6	Cytidine/deoxycytidylate deaminase family protein	
TWT521		−1.5	−1.7		Unknown	
TWT489		−1.5			Unknown	
TWT242		−1.7			HesB/YadR/YfhF family protein	
TWT095		−1.7			Unknown	
TWT194		−1.7			Unknown	
TWT323		−1.7			Unknown	
TWT389		−1.7			Unknown	
TWT656		−1.7			Glycine cleavage T-protein (aminomethyl transferase) superfamily	
TWT572		−1.7			Unknown	
TWT719		−1.7			Unknown	
TWT282		−2			HIT-like protein	
TWT042		−2			Unknown	
TWT596		−2			WISP family protein	
TWT608		−2			WiSP family protein	
TWT716		−2			Putative preprotein translocase SecE subunit	
TWT150		−2			Unknown	
TWT678		−2.5			Unknown	
TWT380		−3.3	−1.7		Unknown	
TWT410		−3.3	−1.7		Unknown	

^a A positive value indicates that the gene is up-regulated during thermal shock, and a negative value indicates that the gene is down-regulated during thermal shock.
^b Gene encoding different functions.

(18.5%), including 44 ORFs having unknown functions, were differentially transcribed compared to the transcription at 37°C ($P < 0.02$); 84 of these genes were up-regulated, and 65 were down-regulated. Genes regulated at 28°C were transcribed in the same way at 4°C but with higher magnitude (Fig. 6 and Table 1; see Fig. S1 in the supplemental material).
The *T. whipplei* genes that were differentially expressed following a 6-h cold shock at 4°C were widely distributed. As shown in

FIG. 2. Comparison of transcription measurements obtained by microarray and real-time RT-PCR assays. The relative transcriptional levels for 11 genes listed in Table 2 were determined by microarray analysis and real-time RT-PCR. The real-time RT-PCR log₂ values were plotted against the microarray log₂ values.

Fig. 3, the major adaptive response was observed for genes involved in fatty acid biosynthesis which were up-regulated, like *bccA* (EC 6.3.4.14) and *fabD* (EC 2.3.1.39), or down-regulated, like *kasA* (EC 2.3.1.41). Genes encoding proteins that are involved in transcription (31.8%) or in nucleoside and nucleotide biosynthesis (25.7%) or have unknown functions (21.8%) were also differentially transcribed, and the numbers of up-regulated and down-regulated genes were comparable. In contrast, 85% and 100% of differentially expressed genes classified in the energy metabolism category and in the central intermediary metabolism category, respectively, were overexpressed. A few sets of genes encoding proteins putatively implicated in amino acid biosynthesis, in DNA metabolism, and in protein synthesis shared transcriptional regulation. A complete regulon composed of five genes having unknown functions (TWT557 to TWT561) was up-regulated. While these genes were not firmly annotated, they putatively encode branched-chain amino acid ABC transporter substrate-binding proteins. Eight regulons (three up-regulated regulons and five down-regulated regulons) were identified by cold shock experiments (Fig. 6; see Fig. S2 in the supplemental material). No searches of conserved DNA motifs upstream of cold shock regulons were successful.

DISCUSSION

***dnaK* operon and virulence factors are up-regulated upon heat shock in *T. whipplei*.** In this study, heat shock was induced by changing the temperature of a bacterial suspension from

TABLE 2. Oligonucleotide primers used for real-time RT-PCR

Gene ID	Gene name	Forward primer sequence/reverse primer sequence (5'→3')	Differential expression temp (°C)
TWT120	<i>tuf</i>	TTATGGAGCTTATGAAAGCCGT/ATTTCAATTCCCGTTACAGTGG	4
TWT441	<i>groEL2</i>	TGGTGCTGATCCTATCAGTTTG/AAGCCGAAAGGTAGCCTTTATC	4
TWT474	<i>mg</i>	GGGTCATAAATCCAGTACCCAA/GCCTACGACGACGATTACCTAC	4/28
TWT481	<i>clpP1</i>	TTGGCTTGGTGAGGCTATAGAT/AAGTAAGCAACACCTGACCCAT	4
TWT675	<i>fusA</i>	TTGTTGATACGGTAACAGGTGG/TGACCTCAACAGACATAATCGG	4
TWT745		TTCAGAAATACTGCCTGCATTG/GGCGAATAATAGCCAGAAAGAA	43
TWT746	<i>clpB</i>	CTTGAAGATGCATCAGCACTTC/TTGGACAAATCCTCCTCAAGAT	43
TWT747	<i>hspR</i>	ACGTACTCTTGGTGGAGACAGG/GCTTTATCCTTGTGCCGTATTC	43
TWT748	<i>cbpA</i>	CAAGACTGGCTTGAGAAGGACT/CTATGGCCCGTGAAGAGATTAC	43
TWT749	<i>grpE</i>	CTCAGATATTCGCTCGGCTAGT/GTGCATCTCTTTCAATCTCAG	43
TWT750	<i>dnaK</i>	TGGAACATTTGACGTTTCACTC/TGGATATTAGCACTGGTTGCAC	43
TWT385	<i>leuS</i>	TAATGAGCAGGTTCTACCGGAT/TTGTAAACACCGTTACAGGTCG	Invariant
TWT523	<i>mgE</i>	TGGAATAACACAACCTGCAAGG/AGTCAGCAGCCTTTATTTCTGG	Invariant
TWT639		AGCACATGTCGTGAGAGTGTTC/CAAGATTTGACGCTAGTGCATC	Invariant

37°C to 43°C over a 15-min period, as reported elsewhere (26). The *T. whipplei* adaptive response was characterized by strong up-regulation of several protein chaperones, like GrpE, HspR, DnaK, CbpA, and the ClpB protease, all of which are very important in the heat shock responses of most known microorganisms (21, 26). The genes encoding these HSP are adjacent and located on the same strand in the *T. whipplei* genome. Their coexpression thus confirms the bioinformatic *dnaK* operon prediction (FGENESB software; Softberry Inc., Mount Kisco, NY). This regulon is composed of *clpB*, *hspR*, *cbpA*, *grpE*, *dnaK*, and TWT745. The last gene, TWT745, which was up-regulated 2.8-fold following heat shock, is an ORFan gene with no homologs in available public databases. These results suggest that TWT745 belongs to the HSP family. In contrast to classical heat shock transcriptional profiles, the two major HSP, GroEL2 and GroES, which are also known to be highly immunogenic and act as early targets in several infectious diseases (61), were not affected. Similar gene expression pro-

files were reported previously for two other reduced-genome bacteria exposed to heat shock, *Buchnera aphidicola* (60) and *Mycoplasma genitalium* (39). As in these bacteria, in *T. whipplei* the percentage of modulated genes in response to heat shock is low. Thus, while *T. whipplei* HSP are up-regulated in a range comparable to that observed for other bacteria exposed to a heat shock, only 19 genes (2.35% of the whole genome) are differentially regulated, compared to 10% of the genes for *Mycoplasma pneumoniae* (58) and *Yersinia pestis* (24) and 15% of the genes for *Shewanella oneidensis* (17) and *B. burgdorferi* (41) (see Table S1 in the supplemental material). This thermal stress was not associated with down-regulation of ribosomal genes, the first step in a decrease in protein synthesis and cell growth arrest. Similarly, metabolic processes were not significantly reduced. Together, these observations suggested that a temperature shift from 37°C to 43°C does not induce a general stress response in *T. whipplei*.

This analysis also highlighted the finding that under heat shock conditions, the *ribC* gene is overexpressed (12-fold increase). This gene is implicated in the biosynthesis pathway for riboflavin (vitamin B₂), a vitamin not synthesized by humans, and is involved in colonization and persistence of *Helicobacter pylori* (13). Bacterial entry into a host cell corresponds to significant environmental changes that are mimicked in part by heat shock, which has been associated with up-regulation of bacterial virulence factors (33).

FIG. 3. Genes differentially expressed at 4°C and 43°C compared to 37°C grouped by functional classification according to The Institute for Genome Research *T. whipplei* genome database (<http://www.tigr.org/>). The percentages of genes were calculated from the number of genes belonging to each functional category, as follows: column 1, amino acid biosynthesis; column 2, biosynthesis of cofactors, prosthetic groups, and carriers; column 3, cell envelope; column 4, cellular processes; column 5, central intermediary metabolism; column 6, DNA metabolism; column 7, energy metabolism; column 8, fatty acid and phospholipid metabolism; column 9, protein fate; column 10, protein synthesis; column 11, purines, pyrimidines, nucleosides, and nucleotides; column 12, regulatory functions; column 13, transcription; column 14, transport and binding proteins; column 15, unknown function.

FIG. 4. Kinetics of *dnaK* and *groEL* transcription for *T. whipplei* exposed to heat shock. The relative levels of transcription of *groEL2* (TWT441) and *dnaK* (TWT750) were determined by real-time RT-PCR using primers listed in Table 2. The data obtained were expressed as the ratio of the values obtained with bacteria exposed to heat shock to the values obtained with untreated cells grown at 37°C. The histograms are representative of two distinct experiments.

FIG. 5. Evidence for two HAIR motifs upstream of the *dnaK* operon in *T. whipplei*. (A) Schematic representation of the *dnaK* operon, including six genes up-regulated with a 15-min heat shock at 43°C. Two homologues of the HAIR motif (18), designated TW_HAIR1 (5'-CATGAGTCGATATGACTCAAT-3') and TW_HAIR2 (5'-CTTGAGTCATTACATGTC AAG-3'), were identified upstream of this region. (B) ClustalW alignment with the HAIR motif initially described for *Mycobacterium tuberculosis* H37Rv (53).

In this respect, we believe that *ribC* could play a role in *T. whipplei* pathogenicity. Other putative virulence factor could be encoded by *ispDF* (3.8-fold increase), which has been described as a potential drug target in several human pathogens, including *H. pylori*, *Campylobacter jejuni*, and *Treponema pallidum* (16). In prokaryotes, heat shock also mimics antibiotic stress. This fits well with enhanced expression of *folE*, a gene involved in folate biosynthesis. Indeed, sulfonamide-sensitive bacteria, like *T. whipplei* (7), must synthesize their own folates (12). Finally, the function of *infC*, which is mainly associated with a cold-sensitive phenotype (23), is unclear.

Evidence for a complex adaptive response of *T. whipplei* to cold shock. In experiments aimed at identifying other putative virulence factors of *T. whipplei* (33), we analyzed the transcriptional variations induced by 6 h of incubation of *T. whipplei* at 28°C. In order to obtain a better understanding of the effect of cold resistance of *T. whipplei*, we also incubated a mid-log bacterial suspension at 4°C for the same time. The results obtained showed that all genes regulated at 28°C were regulated at 4°C but that the magnitude of regulation was higher, indicating that 28°C is lower than the optimal temperature for growth of *T. whipplei* in vitro. Therefore, we did not identify virulence genes which could be down-regulated at this temperature.

Experiments carried out with severe cold conditions (i.e., 4°C) revealed that while *T. whipplei* lacks classical CSP, this microorganism has numerous specific adaptive mechanisms for responding to cold. The most striking feature is the paradoxical up-regulation of the GroEL2 transcript. This transcript encodes a member of the HSP family and is expected to be down-regulated at low temperatures (5, 25). While unconventional, an increase in transcription of HSP60 genes at low temperatures was previously described for the hyperthermoacidophilic archaeon *Sulfolobus shibatae*, which can adapt to and survive in extreme natural environments (30). Another chaperonin-encoding gene, *clpP1*, was also up-regulated, and its role in bacterial tolerance of low temperatures has been suggested previously (14). These HSP were not modified upon heat shock. Whether *T. whipplei* uses these HSP to compensate for a CSP defect is questionable. It could be hypothesized that in *T. whipplei*, such chaperonins ensure pro-

FIG. 6. Circular representation of the *T. whipplei* Twist transcriptome. The outermost (first) circle indicates the nucleotide positions. The second and third circles indicate the ORF locations on the plus and minus strands, respectively. The fourth, fifth, and sixth circles indicate the microarray expression profiles for *T. whipplei* at 4°C versus 37°C, at 28°C versus 37°C, and at 43°C versus 37°C, respectively. The expression profiles for each gene, which are shown with a color-coded, base 2 logarithmic scale, were independently centered about zero. Green indicates decreased expression relative to the expression at 37°C. Red indicates increased expression relative to the expression at 37°C. Yellow indicates no change in expression relative to the expression at 37°C.

tein folding in thermal or acidic environments that are lethal to other microorganisms that have been characterized. Other major changes are related to the fatty acid pathway (*bccA*, *fabD*, *kasA*, *cdsA*), a feature in line with membrane adaptation described for other bacteria exposed to cold. As the physical barrier between living cells and their environment, the plasma membrane is indeed highly susceptible to changes in environmental temperatures. While the lipid bilayers of most organisms are mostly fluid at physiological temperatures, they undergo a reversible change of state at lower temperatures (38). *T. whipplei* probably circumvents alterations in the cell membrane by incorporating fatty acids that have lower melting points in order to restore membrane fluidity and function. Control of the membrane physical properties can also be afforded by observed changes in the transcriptional levels of several genes encoding the WISP proteins, which are specific *T. whipplei* membrane proteins (6).

As reviewed by Gualerzi et al. (23), the so-called "winter shopping list" is highly specific for each bacterial species based on its lifestyle. Thus, analysis of the *T. whipplei* transcriptome at 4°C revealed that there was 1.5-fold or greater up-regulation of several genes involved in energy metabolism, a situation

theoretically encountered when bacteria replicate. Some genes encoding ribosomal proteins (*rpmB*, *rplT*, *rpsN*, and *rplP*) were also up-regulated, a result consistent with the fact that a temperature downshift induces stabilization of secondary structures of nucleic acids, leading to reduced efficiency of mRNA translation and transcription (28). Therefore, other ribosomal proteins encoding genes (*rpsK*, *rpsL*, and *rplK*) were down-regulated to the same extent; the reason for such antagonistic regulation is unclear. Cold shock also promotes down-regulation of *rph* and *mng*, which encode two RNA stabilization factors. At the same time, both *mrc* (RNase III) and *pcnA* [poly(A) polymerase] are up-regulated. RNase III degrades and processes selective RNAs during starvation (2), and poly(A) polymerase synthesizes a poly(A) tail on some specific mRNAs, promoting fast and selective degradation of mRNA-poly(A) (62). In bacteria, RNA degradation permits the cell to scavenge nucleotides for resynthesis of RNAs. We speculate that *T. whippelii* cold shock adaptation is accompanied by repression and activation of key enzymes which act in concert to induce specific changes in gene expression that lead to metabolic adaptation.

Up-regulation of the *purM*, *purC*, *purF*, and *pyrB* genes involved in purine/pyrimidine pathways (*purF* and *purM* form a stimulon) was also observed. Overexpression of these enzymes associated with down-regulation of *prs* leads to a decrease in the 5-phosphoribosyl 1-pyrophosphate pool in *T. whippelii*. In the same nucleotide metabolism pathway (<http://www.genome.jp/kegg/pathway/map/map00230.html>), *deoD* (EC 2.4.2.1), which encodes purine nucleoside phosphorylase, is repressed and might lead to an increase in the amount of guanosine 3',5'-bispyrophosphate (ppGpp). The amount of this alarmone is decreased following temperature downshifts in *Escherichia coli* (29), but up-regulation of alarmone has been reported for *Bacillus subtilis* (57). Whether (p)ppGpp regulation plays a role in the cold shock response remains to be determined.

The *T. whippelii* genome has a large deficiency of genes encoding enzymes involved in amino acid synthesis (44, 46). This explains the finding that, in contrast to what was observed for most bacteria that have been studied (20), the levels of only two genes belonging to this category were increased after a cold shock. A whole up-regulated cold regulon composed of genes classified as genes that encode putative ABC transporters led us to speculate that *T. whippelii* keeps in storage nutrients essential for cold hibernation.

***T. whippelii* dnaK operon is controlled by HspR.** We demonstrated that in *T. whippelii*, the *dnaK* operon and *groEL2* are regulated in opposite ways, and we found two HAIR operator cassettes upstream of *dnaK*. These conserved structural motifs are probably recognized by the HspR repressor also present in the *T. whippelii* *dnaK* regulon. While a few examples of the HspR repressor-HAIR operator system have been described for gram-positive bacteria, such a regulation mechanism is widespread in actinomycetes (18, 52) and has also been found recently in *Deinococcus radiodurans* (49) and *C. jejuni* (1). Of note is the fact that HSP themselves can participate in the regulation process. Thus, autoregulation of DnaK acting in cooperation with HspR was demonstrated in *Streptomyces coelicolor* (8). No other HAIR sites were detected in the *T. whippelii* genome.

Thus, how *T. whippelii* deals with temperature variation

stresses is rather confusing. The data obtained demonstrated that only minor transcriptional modifications regulated by an ancestral negatively regulated mechanism occurred at 43°C. Similar patterns have been reported for both *M. genitalium* (580 kb) (39) and *B. aphidicola* (641 kb) (60). This result is consistent with the fact that, like other reduced-genome bacteria, *T. whippelii* has lost most of the regulatory sequences and protein regulators (34, 44). In this context, the strong cold shock response of *T. whippelii* was striking. We previously observed that *T. whippelii* was highly resistant to freezing and remained viable for 3 years at -80°C without cryoprotective agents (unpublished data). Our results emphasize the fact that microorganisms can exhibit distinct adaptive pathways involving regulatory cascades which are still not known and which may play a role in the survival of reduced-genome bacteria. While gene deletion should be a complementary approach for exploring putative regulatory mechanisms, genetic manipulation of *T. whippelii* has not been successful so far. Therefore, transcriptional analysis of *T. whippelii* (10) is likely to reflect the adaptability of this organism having an environmental origin and could increase our understanding of this deadly and poorly known pathogen.

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