ABSTRACT

MONTERO, CLEMENTE IGNACIO. Molecular microbial ecology of the hyperthermophilic bacterium *Thermotoga maritima*: Transcriptional and physiological response to antibiotic challenge and inter-species interactions. (Under the direction of Robert M. Kelly).

Whole genome cDNA microarray-based analysis was used to monitor differential transcription during cultivation of *T. maritima* under a variety of environmental conditions, including chloramphenicol challenge and in co-culture with other hyperthermophiles. Transcriptional analysis of wild-type *T. maritima* and a resistant mutant revealed mechanisms by which this bacterium responded to chloramphenicol challenge. In the mutant strain, the presence of five mutations in the 23S rRNA, two of which were associated with the catalytic PTC center, were attributed to chloramphenicol resistance. Transcriptional response of *T. maritima* grown in co-culture with *Methanococcus jannaschii*, *Pyrococcus furiosus* or in *P. furiosus* spent media indicated up-regulation of small open reading frames of unknown function. Two of these, TM1316 and TM0504, were examined with respect to their potential ecological roles. The gene encoding TM1316, corresponding to a putative 31 amino acid peptide, was up-regulated during growth in defined media, growth on spent *P. furiosus* media, and when a chloramphenicol-resistant mutant of *T. maritima* was challenged with the antibiotic. In the *T. maritima* genome, TM1316 is located adjacent to a putative S-adenosyl methionine radical super-family processing enzyme and components of a putative ATP binding cassette transporter. The resemblance of TM1316 to Subtilosin A in *Bacillus subtilis*, a known bacteriocin, raised the possibility that this molecule plays a similar role in *T.*

maritima. TM0504, a 42 amino acid peptide previously associated with cell-to-cell signaling and exopolysaccharide production in *T. maritima*, was found to be co-located with the gene for tmRNA, an essential component of the bacterial ribosomal rescue system mediated by trans-translation. A Real Time PCR strategy was developed to detect differential transcription of TM0504 and tmRNA under various growth conditions. Furthermore, bioinformatic analysis of over 200 bacterial genomes indicated the presence of co-located TM0504-like peptides and tmRNA. Taken together the results from this study demonstrate the strategic use of DNA microarrays and global transcriptional response analysis to reveal otherwise inaccessible features of molecular microbial ecology.

MOLECULAR MICROBIAL ECOLOGY OF THE HYPERTHERMOPHILIC BACTERIUM *THERMOTOGA MARITIMA*: TRANSCRIPTIONAL AND PHYSIOLOGICAL RESPONSE TO ANTIBIOTIC CHALLENGE AND INTER-SPECIES INTERACTIONS

by

CLEMENTE IGNACIO MONTERO

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APPROVED BY:

Dr. James W. Brown

Dr. Amy Grunden

Dr. Todd R. Klaenhammer

Dr. Eric S. Miller

Dr. Robert M. Kelly
Chair of Advisory Committee

Dedication

The work and efforts summarized in this dissertation are dedicated to my beloved wife and my adorable daughter Isabel. Thanks for the inspiration and support during so many difficult times.

Clemente Ignacio.

Biography

Clemente Ignacio Montero was born to Marie Josephine and Clemente Antonio Montero on March 2, 1974 in Caracas, Venezuela. He and his three siblings grew up in Maracaibo, Venezuela. After graduating from High School, Clemente attended the Universidad Simon Bolivar in Caracas. As an undergraduate he worked under the guidance of Dr. Howard Takiff in the characterization of mechanisms of resistance in *Mycobacteria.* He received a Bachelor of Science degree in Biology in 1998 and accepted a research position which focused on the development of phage-based drug susceptibility kits for diagnostic applications. In the spring of 2000 he began his studies in the Microbiology PhD Program at North Carolina State University under the guidance of Dr. Robert M. Kelly and learned how to implement high-throughput approaches for the study of the physiology of hyperthermophilic bacteria. During this time, Clemente married Cristina Carolina Romero. On August 2nd 2005 he became a proud Dad, of a beautiful baby girl named Isabel. After completion of his degree, he plans to take a vacation in Venezuela before beginning a post-doctoral clinical microbiology fellow at the National Institute of Health in Bethesda, Maryland.

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MOLECULAR MICROBIAL ECOLOGY OF THE HYPERTHERMOPHILIC BACTERIUM *THERMOTOGA MARITIMA*:

TRANSCRIPTIONAL AND PHYSIOLOGICAL RESPONSE TO ANTIBIOTIC CHALLENGE AND INTER-SPECIES INTERACTIONS

OVERVIEW

Laboratory studies of pure microbial cultures have provided considerable information about genetic regulation, metabolic capabilities, physiological characteristics and biotechnological potential. However, in nature, bacteria are never “alone”. They exist in their natural environments, competing with other microorganisms for nutritional sources, establishing in many cases intricate metabolic interdependencies for the utilization of nutritional sources available in their environment (26). These interactions control the processing rates that drive the biogeochemical cycles that sustain the biosphere (21). The available tools for study of microbial behaviors in natural environments are limited, with most oriented to the creation of a semi-quantitative microbiological inventories or to providing evidence of metabolic fluxes based on the assimilation of a radioisotope (1, 4, 17). An increasingly popular alternative, the “metagenomics” approach, consists of the collection of massive amounts of DNA sequences from culturable and unculturable microbes inhabiting a specific environment (24). This approach leans heavily on computer-based informational processes that are constrained by the availability and validity of genomic sequence database entries (23). Although this approach contributes to the understanding of the genetic diversity of a

population structure, it does not contribute to the development of a corresponding functional database (23).

In our laboratory, we have applied what has been described as “Ecological genomics” (8) to the study of hyperthermophile physiology and molecular microbial ecology. Basically, it is the application of transcription profiling tools for the study of ecological interactions to provide insights into the evolution of gene usage or the interactions of specific microorganisms within its environment. Chapter 1: “Transcriptomes of the hyperthermophiles *Pyrococcus furiosus* and *Thermotoga maritima* are influenced by co-cultivation and growth in each other’s spent media” describes the simultaneous transcriptional profiles of these two hyperthermophilic, strictly anaerobic heterotrophs in co-culture, used as a simplified model for the study of ecological interactions of microorganisms in their natural environments. Presented in Chapter 1 is a “proof of concept” that can be expanded for implementation with many other microbial groups. This approach is based on the fact that comparative genome hybridization analysis indicates that probes with less than 85% nucleotide sequence identity can be differentiated during transcriptional profiling (18). This implies that the potential for cross-hybridizations for DNA microarray analysis of co-cultures from distantly related species should be minimal, which was demonstrated for the two hyperthermophiles studied here. We took advantage of the availability of two full genome microarray platforms for *T. maritima* and *P. furiosus*, hyperthermophilic microorganisms isolated by Karl Stetter and colleagues from hydrothermal waters near Vulcano Island, Italy (7, 11). *T. maritima*, a bacterium that possesses versatile carbohydrate utilization capabilities, by virtue of lateral gene transfer events noted within

its genome, displays genomic features that resemble archaeal species (19, 20, 31). For example, reported lateral gene transfer events include DNA from an unknown microorganism related to the family of the *Pyrococcales* (31). *P. furiosus* is an archaeon capable of growth at temperatures higher than 90°C and able to utilize a range of poly- and oligosaccharide substrates for growth (2, 6, 7). The fact than *T. maritima* and *P. furiosus* belong to different kingdoms of life make them ideal candidates for studying multi-species interactions at the gene regulation level. This approach not only provides an insight into inter-species interactions, but also contributes to the functional characterization of genes present in each microorganism. The present approach enabled discovery of biological responses otherwise inaccessible in pure culture conditions. This strategy could be implemented and expanded for study of microbial interactions between other Archaea and Bacteria or perhaps for phylogenetically-related species if strategic design of gene probes can avoid interference.

Despite the perception that Archaea are extremophiles adapted to harsh conditions, it is now recognized that they are a diverse and widespread, having been found in forests, freshwater lakes, in the ocean's plankton, sediment and subsurface (see (37) and references therein). In fact, nitrifying marine archaea have been identified as important elements in the global carbon and nitrogen cycles (34). Furthermore, in some cases, their ecological role can be connected to archaeal/bacterial consortia that, for example, have been implicated in the methane oxidation in ocean sediment (33). Therefore, the implementation of DNA microarray technology may further enhance our understanding of these processes and further understanding of how genotype relates to phenotype in environmental settings. Understanding interactions in co-cultures provides a

good starting point to more complex studies, with the final goal of understanding microbial interactions in mixed communities under conditions that reflect their natural environments.

We observed in our *T. maritima/P. furiosus* platform that both the cell density and media composition had profound influences in the transcriptional profiles of these two microorganisms during co-culture. Unexpectedly, the up-regulation of a previously uncharacterized operon including the locus TM1316-TM1319 was detected. This directed our attention to features in this operon that resembles bacteriocin-like cassettes present in mesophilic bacteria. However, is important to emphasize that similarities at the level of the genomic organization or at the level of the structures present in these small putative antimicrobial peptide does not necessarily imply an antibiotic role. A quite interesting example of this is provided by the existence of a diffusible signal produced by *Streptomyces coelicolor* (15). This signal SapB is associated with the morphogenesis of aerial hyphae and sporulation. It is encoded by a RamS which is proteolitically processed, structurally SapB resembles type A lantibiotic peptides (5, 25). Although SapB has two intramolecular loops formed by the dehydration of Serine or Threonine residues. It lacks the conventional the N-terminal leader sequence commonly found in L-antibiotics (15). Instead, the putative RamS leader peptide is not particularly hydrophilic, and it lacks both of the two characteristic cleavage sites. The RamS leader peptide resembles that of the type A lantibiotics only in that it possesses a high proportion of charged amino acids and has a net charge of -3 (15). Based on the genomic organization, a dedicated ABC transporter seems to be associated with this molecule which does not have antimicrobial properties and rather functions as a surfactant and as an accessory signal molecule during

morphogenesis (15). However, it is known that antimicrobial peptides exist across all domains of life (28, 29) and considering the important proportion of microbial biomass potentially associated in geothermally-heated environments and earth's sub-surface (9), it is a virtual certainty that antagonistic small molecule mediated relationships in hyperthermophilic environments exist. In fact, such interactions have been documented in thermophilic environments. For example, peptide antimicrobials have been characterized in *Halobacterium sp.* that are toxic to *Sulfolobus solfataricus* and *S. shibatae* (10). However, responses to the presence of antimicrobials in hyperthermophilic environments are a virtually unexplored subject.

In the isolation report for *T. maritima*, it was found that this hyperthermophilic bacterium was sensitive to the translational inhibitor chloramphenicol (11). Chloramphenicol is in fact a candidate for a selective marker in the development of systems for gene delivery, which are not available for this microbe (32). Responses to this translational inhibitor have been studied in several bacteria, and it has been recognized that they present common features at the transcriptional level that are conserved across Gram-positive and Gram-negative bacteria (3, 12, 16, 22). In Chapter 2 "Response of wild-type and resistant-mutant strains of the hyperthermophilic bacterium *T. maritima* to chloramphenicol challenge," we present the transcriptional response profiles of *T. maritima* and a resistant mutant strain to chloramphenicol challenge in both batch and continuous cultures. The potential mechanisms of resistance are discussed as well as the genomic locations of mutations in the 23S rRNA linked to resistance to chloramphenicol. The transcriptional response in some conditions showed transcriptional

regulation of tmRNA, an essential component in the process of ribosomal rescue in bacteria mediated by trans-translation (14, 30)

Recently, it has been found that a signaling peptide TM0504 is implicated in the synthesis of exopolysaccharides by *T. maritima* in co-culture with the hyperthermophilic methanogen *M. janaschii* (13). That was co-located within the complementary strand of the tmRNA gene. Chapter 3: "Transcriptional analysis of co-located ORFs encoding a signaling peptide (TM0504) and tmRNA in the genome of the hyperthermophilic bacterium *Thermotoga maritima*" addresses this topic. It discusses the possibility of similar mechanisms in other bacteria; analysis of 200 genomes provides evidence that this particular conformation might be present in other bacterial groups. We found that in some cases the genes neighboring tmRNA suggest a functional role similar to the one previously described in *T. maritima* (13). Discussed in this chapter is a novel Real-time-PCR method that was employed to study the differential transcription of the genes encoding tmRNA and TM0504.

The existence in the same genetic locus of an overlapping regulatory/physiological relevant RNA molecule and a protein in the complementary strand expand the already convoluted definition of a gene (27) and raises interesting issues regarding the origin and conservation of these two co-located genes. Clearly, these two genes are evolutionarily linked, because a mutation in one of them has the potential to affect the other gene. Therefore, mutations in this particular locus would occur less often than the overall mutation rate of any given bacteria. In this case it will be interesting to investigate the relationship between the constraints associated with the need for a functional RNA sequence requiring a terminal 3' that needs to be aminoacylated

(14, 30) and the need on the complementary strand of a species specific signaling protein molecule.

The present document provides a clear example that the integration of transcriptional profiling and comparative functional genomics provides valuable information to address the functional roles of biomolecules encoded in the genomes of hyperthermophilic microorganisms. This approach was put to strategic use here to understand the molecular microbial ecology of *T. maritima* and this approach can be extended further to study the complexity of less thermophilic microbial interactions.

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CHAPTER 1:

Transcriptomes of the hyperthermophiles *Pyrococcus furiosus* and *Thermotoga maritima* are influenced by co-cultivation and growth in each other's spent media

Clemente I. Montero, Derrick L. Lewis, Steven R. Gray, Matthew R. Johnson, Elizabeth A. Nance, Shannon B. Conners, Keith R. Shockley, and Robert M. Kelly

Department of Chemical and Biomolecular Engineering
North Carolina State University
Raleigh, NC 27695-7905

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ABSTRACT

Whole genome cDNA microarrays, developed for the hyperthermophiles *Pyrococcus furiosus* and *Thermotoga maritima*, were used to examine the influence of growth in co-culture and in each other's spent media on their respective transcriptomes. Only minimal cross-hybridization was noted such that transcriptional analysis for each microorganism in the presence of the other was not compromised. In co-culture, transcriptional response was a function of the extent of growth. In high peptide medium (3 g/l yeast extract and 5 g/l tryptone), when sampled in early exponential phase (approximately 10^7 cells/ml for each microorganism), 64 ORFs (36 up/28 down) were differentially expressed 2-fold or more in *P. furiosus* compared to pure culture, while 334 ORFs (189 up/145 down) were affected at this level in *T. maritima*. When sampled in late exponential phase (approximately 10^8 cells/ml for each microorganism), *T. maritima* (56 ORFs, 20 up/36 down) was less affected than *P. furiosus* (451 ORFs, 212 up/239 down), compared to pure cultures of each microorganism. Among the ORFs up-regulated in organism's transcriptome were several small (<120 amino acids) putative peptides. In low peptide medium (1 g/l yeast extract and no tryptone), limited differential expression was noted for each hyperthermophile sampled in early exponential phase (10^7 cells/ml) compared to pure cultures, with 68 ORFs (25 up/43 down) affected 2-fold or more in *P. furiosus* compared to 38 ORFs (16 up/22 down) in *T. maritima*. Both hyperthermophiles were also cultured in each other's spent low peptide medium and sampled in early exponential phase. When *P. furiosus* was grown in spent *T. maritima* low peptide medium, differential expression of 108 ORFs (53 up/55 down) was noted. When *T. maritima* was grown as a pure culture in *P. furiosus* low peptide, spent medium, 39 ORFs were

differentially expressed (34 up/5 down) 2-fold or more. Many of the up-regulated ORFs in *T. maritima* during growth in *P. furiosus* spent medium belonged to a locus (TM1300-TM1338), which included a putative peptide, TM1316, evidence for which was previously detected in the *T. maritima* secretome and the corresponding gene for which was differentially expressed during chloramphenicol challenge. Bioinformatic analysis revealed that TM1316 had features related to a known cyclic, anti-listerial bacteriocin, subtilosin A, produced by *Bacillus subtilis*. Comparison of the operon containing TM1316 in *T. maritima* and the subtilosin operon in *B. subtilus* revealed the presence of a putative SAM radical superfamily processing enzyme (TM1317), belonging to the same COG as *B. subtilis* AlbA, implicated in the post-translational cyclization of Subtilosin A. Further work is needed to determine the role of TM1316 in *T. maritima* and its possible relationship to previously studied bacteriocins. Nonetheless, the results presented here demonstrate that multi-species interactions and other ecological behaviors can be probed using species-specific DNA microarrays and that phenomena otherwise inaccessible can be examined using this functional genomics approach.

INTRODUCTION

Molecular microbial ecology focuses on understanding mechanistic relationships between habitat characteristics, microbial diversity, microbial processes, and underlying genetic controls (23). However, advances in this field have been limited due to the complexity of systems studied and the physical, geochemical, temporal and microbial diversity associated with them (48, 62). The recent implementation of molecular biology tools and refinement of traditional culture-dependent approaches have facilitated understanding of the structure of microbial communities (48, 62). For example, the utilization of diffusion growth chambers have enabled, not only the isolation of "non-cultivable" strains, but also identification of ecological interactions based on extracellular signals (5, 6, 24, 25, 32). Gel-caged micro-colonies have also been used for the study of potential cross-feeding and signaling between uncharacterized microbes (86). Complementary cultivation-independent strategies, such as 16S rRNA phylotyping (59, 73), fluorescence *in situ* hybridization (FISH) (17, 22), denaturing gradient gel electrophoresis (DGGE) (55), restriction fragment gel polymorphism (RFLP) (44, 54), DNA microarrays (3, 7, 42, 69), and environmental clone libraries or "metagenomic" approaches (60), have been used to assess diversity in a wide range of microbial communities. However, there can be problems with these approaches (34, 48, 79). Their implementation is limited to indirectly answering the question of what function and traits are associated with a specific microorganism in an environmental setting and ultimately requires ecological validation which, in most cases, is difficult to do (34, 48).

Microarray technology has been implemented for identification of individual species in microbial communities and used as a high throughput extension of well-

established approaches such as 16S rRNA phylotyping (34). This approach has been used in applications that range from clinical diagnostics to the screening of microbial populations in soil and aquatic environments (7, 42, 69, 88). Recently, the use of DNA microarrays has emerged as a promising approach for studying functional aspects of individual species within complex microbial communities. For example, microarrays have been used to describe the response of human gastric cells to *Helicobacter pylori* (80, 84, 85), as well as the transcriptional response of *Porphyromonas gingivalis* to human epithelial cells (28) and in co-culture biofilms with *Treponema denticola* (37). Fungal and bacterial antagonistic interactions have been examined using the antifungal microbe *Bacillus lentimorbus* WJ5 and its response upon exposure to the phytopathogen *Colletotrichum gloeosporioides* (41). Microarrays have also been used to probe syntrophic relationships between hyperthermophilic archaea and bacteria, such as *Methanococcus jannaschii* and *Thermotoga maritima* (31).

In all the aforementioned studies, the relationship of transcriptional response to ecological interaction remains incomplete because this information is obtained for only one of the species present. However, if cross-species hybridization can be minimized through strategic design of probes, species-specific DNA microarrays could be used to detect the genetic basis for ecological interactions and the regulatory basis for the resulting phenotypes. To this end, we describe here an approach for obtaining simultaneous, whole genome transcriptional responses for two microorganisms cultured together, providing insights otherwise inaccessible using other approaches. The focus was on a hyperthermophilic bacterium, *Thermotoga maritima*, and a hyperthermophilic archaeon, *Pyrococcus furiosus*, microorganisms originally isolated from hydrothermal

waters near Vulcano Island, in Italy (19, 29), and frequently co-isolated from similar environments (46, 61, 75). Beyond the use here of this technique for examining hyperthermophile microbial ecology, it can be adapted and applied for study of microbial interactions in less thermophilic settings.

MATERIALS AND METHODS

Growth of microorganisms. *T. maritima* strain MSB8 (T_{opt} 80°C) and *Pyrococcus furiosus* DSM 3638 (T_{opt} 98°C) were grown anaerobically at 80°C on media with and without tryptone (31, 65). Tryptone-based media (or high peptide media, HPM) consisted of 40 g/l sea salts, 5 g/l tryptone, 3 g/l yeast extract, 3 g/l Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES), 0.5 mg/l $NaSeO_3 \bullet 5H_2O$, 0.5 mg/l $NiCl_2 \bullet 6H_2O$, 0.25 g/l NH_4Cl, 10 mg/l $Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$, 1.25 g/l sodium acetate anhydrate, 1 ml/l 1% Resazurin, 10 ml/l Wolfe's trace elements and 10 ml/l Wolfe's trace vitamins solution (composition of these vitamin and minerals solutions corresponds to ATCC medium no. 1343). Tryptone-free media (or low peptide media, LPM) media contained 40 g/l sea salts, 1 g/l yeast extract, 3.1 g/l PIPES buffer, 2 ml/l of 0.05% Resazurin, and 10 ml/l 10X Wolin minerals. Both media were adjusted to pH 7.0 and 6.8, respectively, prior to autoclaving. Before inoculation, each medium was supplemented to 10 mM with cellobiose that had been filter-sterilized. All media components were obtained from Sigma Chemical (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Batch cultures (50 ml) were inoculated under N_2 (high purity nitrogen; National Welders, Raleigh, NC) headspace, as previously described (65); a solution of 10%

sodium sulfide:10% l-cysteine HCl (pH 8.0) added to a final concentration of 0.05% was used to establish reducing conditions. At least three passages were done in either LPM or HPM prior to inoculation for transcriptional response measurements. Cultures were inoculated at 1% and grown in a 3l Applikon glass bioreactor with a 2l working volume. A BioConsole ADI 1025 (Applikon, Foster City, CA) was used to control N_2 sparging at 100 ml/min and agitation at 200 rpm. Glutaraldehyde-fixed culture samples were stained with acridine orange for cell enumeration, as previously described (30). Differences in cell morphology between the rod-shaped bacterium *T. maritima* and the coccoid-shaped archaeon *P. furious* were used discriminate between the microorganisms during co-culture experiments (See Figure 1.1).

For co-culture experiments in HPM at 80°C, a culture of *P. furiosus* (T_{opt} of 98-100°C) was first established and allowed to reach 10^7 cells/ml before a 1% inoculum of *T. maritima* was added. For the establishment of co-cultures in LPM, a *P. furiosus* culture was inoculated with *T. maritima* at 1% as soon as *P. furiosus* reached 5 x 10^6 cells/ml. Cultures were harvested when each organism reached either approximately 10^7 or 10^8 cells/ml. For spent media experiments, cells were initially grown to approximately 10^7 cells/ml, then harvested and centrifuged at 13,000 x g for 30 min at 4°C. The resulting supernatants were filter-sterilized twice with a 0.22 μm Durapore membranes (Millipore, Billerica, MA.) and stored at 4°C until use within 24 h. No adjustment in pH, nor addition of cellobiose or reducing agent was done. Anaerobic conditions were established prior to inoculation by sparging with N_2. See Table 1.1 for a summary of the experimental conditions tested.

Microarray protocols. Construction of whole genome *T. maritima* and *P. furiosus* microarrays have been described elsewhere (10, 65, 71). For each experimental condition, RNA was obtained from 350 ml samples that were harvested and immediately cooled to 0°C by immersion in ice water. The cell pellets obtained by centrifugation at 13,000 x g (15 min at 4°C) were used to extract total RNA, following a protocol described previously (20). First-strand cDNA was generated using Superscript III and random primers (Invitrogen, Carlsbad, CA) and labeled indirectly with amino-allyl (Ambion, Austin, TX). Hybridizations were performed following methods described elsewhere (65, 70). Balanced raw intensities was obtained using an ExpressLite Scanner and quantification was performed using ScanArray (Perkin-Elmer, Freemont, CA.). Data analysis was done as discussed previously (10, 12). Given the replication of treatments, arrays, dyes and cDNA spots, it was possible to implement an ANOVA model based on a loop design for reciprocally labeled hybridizations (35, 36). Briefly, linear normalization was used to assess global variation and to differentiate the least-squares mean estimates, of gene-specific treatment effects, from random and systematic errors (81). Pair-wise comparison of least-square means were represented as contrasts or fold changes between treatments (81). Visualization of interesting contrasts was done with Volcano plots (81). Unless otherwise noted, original gene annotations were confirmed against the COG database at NCBI (76) and the Conserved Domain Database at NCBI (49). Information on the magnitude and statistical significance of fold changes for all of the genes included on the arrays, will be available at http://www.che.ncsu.edu/extremophiles/.

For cross-hybridization experiments, reciprocal labeling (dye-flip) was performed using cDNA from reference conditions: pure culture of *T. maritima* and *P. furiosus*

grown on HPM tested against the microarray platforms of *P. furiosus* and *T. maritima*, respectively. Data acquisition was done at the scanner's full laser power and a photomultiplier tube gain of 70%. Signal intensities and signal-to-noise ratios were obtained directly from the Scanarray Express software. Mummer (16) and tools for comparative genomic analysis available at the CMR site (64) were used for alignment and interpretation of cross-hybridization results.

RESULTS and DISCUSSION

Cross species hybridization. The primary objective here was to determine if microbial behaviors in co-culture could be examined through simultaneous transcriptional response of the two microorganisms using species-specific DNA microarrays. The focus was on two heterotrophic hyperthermophiles, the bacterium *T. maritima* and the archaeon *P. furiosus*, that were originally isolated from a similar site near Volcano Island, Italy (19, 29). A key issue was whether cross-hybridization between the species of interest would interfere with transcriptional analysis. Previously, when interrogated with cDNA from the archaeon *M. jannaschii*, the *T. maritima* microarray platform showed no evidence of cross-hybridization (30, 31). Using cDNA from *T. maritima* labeled with both Cy5 and Cy3 dyes and scanning at maximum laser power, no cross-hybridization with the *P. furiosus* platform was observed. When *P. furiosus* cDNA was tested with the *T. maritima* platform, a weak cross hybridization signal was observed. The average signal to noise ratio (SNR) of the spots when labeled *P. furiosus* cDNA was used against the *T. maritima* platform was 157, with a maximum value of

312. On the other hand, the control *T. maritima* cDNA generated an average SNR of 1184 with a maximum of 2360.

Genes with the highest SNR for *P. furiosus* cDNA on the *T. maritima* platform were further investigated. Out of 1927 probes on the *T. maritima* array, 54 had average signal intensities corresponding to more than 50% of the reference *T. maritima* cDNA hybridizations. These 54 probes consistently ranked in the lowest 10^{th} percentile for more than 12 experimental conditions examined with *T. maritima* pure cultures (data not shown). Since lateral gene transfer has been implicated in the evolution of *T. maritima*, connections with *P. furiosus* along these lines cannot be ruled out (57, 82). However, genomic alignments at the nucleotide level, using the software tool Mummer, were able to account for possible cross-hybridization of only 3 of the 54 aforementioned genes. Furthermore, alignment tools available at the comprehensive microbial resource (64) also failed to explain the weak cross-hybridization observed. For these reasons and the consistently low signal intensities of these probes, they were eliminated from this analysis. Recent studies of comparative genomic hybridizations in *Thermotoga sp.* using similar experimental conditions showed that no significant cross-hybridization was observed as long as the species to be compared had less than 85% identity at the nucleotide level (53). Other studies focusing on environmental samples indicate the need for more stringent hybridization conditions for resolution of genes with 80 to 85% identity (83).

Transcriptional response of *P. furiosus* in co-culture with *T. maritima* growing in HPM at 80°C. As shown on the Tables 1.2-1.4 and Figure 1.2, only 64 genes

were differentially expressed in *P. furiosus* co-cultured with *T. maritima* compared to pure culture at low cell densities (10^7 cells/ml). Among the 36 genes up-regulated, several small ORFs of unknown function (see Tables 1.4 and 1.10) were noted. The transcriptional response in *P. furiosus* associated with co-culture was mainly characterized by the up-regulation of genes associated with the TCA cycle and metabolic incorporation of nitrogen, including genes encoding: aconitase A (AcnA) (PF0201, +2.9-fold), isocitrate dehydrogenase (Icd) (PF0202, +2.6-fold), citrate synthase (GltA) (PF0203, +2.7-fold), glutamate synthase subunit 1 (GltB) (PF0204, +2.0-fold), and glutamate synthase subunit 2 (GltC) (PF0205, +2.0-fold). Also, up-regulation of genes associated with leucine biosynthesis was detected: 2-isopropyl-malate synthase LeuA (PF1678, +3.4-fold), 2-isopropyl-malate dehydrogenase (LeuC) (PF1679, +3.4 fold), and isopropyl malate dehydrogenase (LeuD) (PF1680, +3.8-fold) was detected. On the other hand, in co-culture, POR, linked to the production of pyruvate, acetyl-CoA, CO_2 and acetataldehyde (47) and encoded by (PF1768-PF1773), was down-regulated -2.4-fold. Interestingly, a second operon flanked by PF0935 and PF0943, associated with the synthesis of leucine, was down-regulated -2.5 fold.

At high cell densities (10^8 cells/ml) in the co-culture (see Table 1.2, Figure 1.3, and supplementary Table 1.8), transcriptional response of *P. furiosus* to the presence of *T. maritima* was significant. A total of 451 genes were differentially expressed (212 up/239 down). Among the up-regulated genes in *P. furiosus* in co-culture (Table 1.8) were two aminopeptidases (PF2065, PF2059), along with a transporter component and transcriptional regulator (PF2061 and PF2062). Pyrolysin (PF0287) (+3.8 fold change), as well as two Xaa-Pro peptidases: PF0746 (+2.1 fold) and PF0702 (+2.0 fold), were up-

regulated. Up-regulation (+2.6-fold) of the genes encoding the Mnh Na/H+ anti-porter (PF1423-PF1429) was observed. The adjacent genes that belong to the Nuo1 membrane-bound dehydrogenase (PF1436-PF1456) were differentially expressed +3.0-fold. It was interesting that down-regulation of 90% of ribosomal proteins was noted, as well as genes involved in the synthesis of glutamine, arginine, asparagine, threonine, histidine, isoleucine and proline. Unlike the case for *T. maritima*, only marginal differences were observed in specific growth rate and final cell yield of *P. furiosus* in co-culture compared to pure culture.

Transcriptional response of *T. maritima* in co-culture with *P. furiosus* growing in HPM at 80°C. When grown in co-culture with *P. furiosus* in HPM at 80°C, *T. maritima* grew at a higher rate than for the same conditions in pure culture (doubling time of 53-60 min compared to 123 min). When the co-culture was sampled at 10^7 cells/ml (see Table 1.2), 334 genes were differentially expressed in *T. maritima* (214 up-regulated/120 genes down-regulated), compared to a pure *T. maritima* culture grown under the same conditions (see Figure 1.3). In total, 59 ORFs associated with ribosomal protein operons were differentially transcribed, corresponding to 71% of all ribosomal proteins annotated in the *T. maritima* genome sequence, consistent with the notion that growth rates and concentrations of ribosomal proteins are directly correlated (58). Other indications of increased growth were observed in *T. maritima* in the co-culture, including up-regulation of FtsZ (TM0836, +2.1 fold), Era (TM0847, +2.8 fold) and MrpB (TM1593, 2.5 fold), all of which are implicated in cell division. The tubulin-like FtsZ protein forms the cytokinetic ring at mid-cell and thereby provides a scaffold on which

other essential cell division proteins can assemble (50). MrpB is a homolog of ParA, which is known to facilitate chromosome partition in bacteria and plasmids (2). Era, also is implicated in the control of the cell cycle progression and its depletion is associated with the lack of nucleoid segregation (4). Differential expression of essential cell division genes was coupled with the up-regulation of genes involved in *de novo* synthesis of deoxynucleotides, such as GuaB (TM1347- inositol monophosphate dehydrogenase, +2.8 fold), Cmk (TM1443-cytidilate kinase, +3.9 fold), Adk (TM1479-adenylate kinase, +4.9 fold), Gmk (TM1689-guanylate kinase, +2.0 fold), and PrsA (TM1628-phosphoribosyl pyrophosphate synthase). Likewise, up-regulation of TM0025, a putative β-D-glucoside glucohydrolase, transcriptionally responsive to β-glucans, was indicative of increased growth substrate processing (12). In addition, several genes that correspond to functions associated with energy production and conversion, such as pyruvate ferredoxin oxidoreductase (POR) subunits TM0016 (+2.5 fold), TM0017 (+2.5 fold), and TM0018 (+2.1 fold), PykF (TM0208-pyruvate kinase, +9.9 fold), PfkA (TM0209-6-phosphofructokinase, +3.9 fold), Pgk (TM0689 phosphoglycerate kinase, +3.0 fold) and Eno (TM0877-enolase, +2.1 fold), were also up-regulated, as were components of the TCA cycle SdhA (TM1148, +2.3 fold), IcdA (TM0202, succinate and isocitrate dehydrogenases, respectively. It is not clear what triggered growth-intensive genes in *T. maritima* in co-culture, although synergism in which extracellular hydrolytic enzymes of *P. furiosus* complement those produced by *T. maritima* remains a possibility. Previous reports demonstrated such synergism in the degradation of complex sugars by *P. furiosus* (18). Given the exceptional versatility of *T. maritima* in the utilization of a wide variety of sugar substrates (12), it may also be that bioavailability of the peptide/amino acid

substrates in the media is increased by actions of *P. furiosus* in the co-culture. It was interesting that up-regulation of genes associated with protein turnover and chaperones in *T. maritima* was noted in the co-culture, including: ClpX (TM0146, +2.5 fold), ClpA (TM0198, +2.8 fold), GroES (TM0505, +2.5 fold), GroEL (TM0506, +4.0 fold), HslU (TM0522, +2.8 fold), Tig (TM0694, +5.7 fold), SurA (TM0912, +2.1 fold), ClpA (TM1391, +2.1 fold), Lon (TM1633, +3.5 fold), SurA1 (TM1704, +2.1 fold) and ClpP, (TM0695, +3.0 fold), respectively. In addition, ORFs associated with chemotaxis and exopolysaccharide synthesis (see Table 1.3) were up-regulated in the co-culture. Glycosyltransferases responsive in co-culture with *P. furiosus,* TM0627 and TM0631, were previously implicated in the formation of cellular aggregates in synthrophic co-culture with *M. jannaschii* (31).

The contrast between the transcriptional profiles of *T. maritima* pure cultures and co-cultures, both at 10^7 cells/ml, also included 120 genes that were down-regulated in the co-culture (see Tables 1.2 and 1.11). Among them were many genes associated with transport and utilization of sugar substrates other than cellobiose. Down-regulation was noted for TM0057-60 (average –2.0 fold), components of a putative ABC transporter, and TM0070 (endo-1,4-β-xylanase, -2.3 fold), previously associated with xylan and xylose utilization (12). TM0810 (-2.1 fold), TM0811 (-2.6 fold), TM0595 (-2.3 fold), components of putative transporters that were previously shown to be responsive to the presence on chitin or N-acetyl-glucosamine (12), as well as genes responsive to the presence of rhamnose TM1063 (-2.0 fold), TM1064 (-2.1 fold), and TM1067 (-2.1 fold), were all down-regulated in the co-culture condition. Down-regulation of TM0729 (-2.8 fold), annotated as (p)ppGpp synthase which is related to the RelA/SpoT family of

proteins (52) was observed; whether this relates to some form of stringent response such as that observed in *E. coli* (9) is not known.

Several small genes of unknown function responded in *T. maritima* in co-culture with *P. furiosus* (see Table 1.4). For some of these, this is the first instance of differential transcription from among more than 50 different experimental conditions that have examined for *T. maritima* cultures in our lab (data not shown). At this point the role of these small genes is uncertain, but some might encode small RNAs (45, 74).

A complementary co-culture experiment was done focusing on the transcriptome of *T. maritima* when both microorganisms reached 10^8 cells/ml. In this case, only 56 genes (20 up/56 down) in *T. maritima* were differentially expressed when compared to pure culture of *T. maritima* grown to the same cell density. Among the 20 genes being up-regulated in the co-culture only two 0035 and TM1685) showed the same pattern of differential expression when compared to the low cell density experiment: TM0035, a hypothetical protein without any recognizable protein domain according to the Smart Database, and TM1685, which has RNA binding domains, VacB and S1 (43), and is 28% identical at the amino acid level to BSU31390, a stress response protein previously identified in *Bacillus subtilis* (1). The remaining genes that were up-regulated in the co-culture have poorly characterized functional annotations (76), a few of which are associated with deoxynucleotide metabolism: phosphoribosylglycinamide synthase (TM1246, +2.0-fold), ribonucleoside triphosphate reductase (TM0385, +2.6-fold), and a predicted nucleotide kinase (TM0036, +2.0-fold). Three genes were down-regulated in HPM harvested at low and high cell density, including TM1232, an ATP-binding component of a transporter responsive to the presence of maltose. The other genes that

werely “lower” under both conditions were TM0338 and TM0826, both of which correspond to small hypothetical peptides of 35 and 56 residues, respectively. According to ProtParam, these are stable proteins with a pI higher than 9.0 (21). TM0338 is flanked by TM0337, a SAM (S-adenosyl methionine) Radical super-family protein containing an Elp3 domain (43), and TM0339, an uncharacterized protein belonging to COG03877 (76). Curiously, TM0826 is flanked by TM0825, another SAM-radical super family protein and member of the AstB/ChuR family, and TM0827, a putative ABC transporter and ATP-binding protein. We did not observe differential expression of either TM0825 or TM0827, but did for TM0824, which is also a putative AstB/ChuR-related protein. A more detailed description on the possible role of these AstB/ChuR proteins adjacent to small open reading frames is provided below. For consistency with the annotation in the *T. maritima* genome we will keep the designation of AstB/ChuR, however as explained in the appendix B a more appropriate designation should be adopted, either AslB/ChuR or AtsB/ChuR.

Transcriptional response of *P. furiosus* in co-culture with *T. maritima* growing in LPM at 80°C. Defined media formulations have been described for both *T. maritima* and *Pyrococcus sp.* (66, 68). However, adapting these media for growth of *P. furiosus* DSM3638 was not successful. As a consequence, an undefined low peptide medium (LPM) that contained only 1 g/l of yeast extract and no tryptone was developed that supported the growth of both hyperthermophiles at 80°C. This was used to examine the effect of co-culture on transcriptomes for cell densities at approximately 10^7 cells/ml in the presence of low levels of growth medium-based peptides. Shown in Figure 1.4 is

the growth of *T. maritima* and *P. furiosus* in co-culture in LPM. Table 1.2 shows that only 38 genes (16 up/22 down) were differentially expressed 2-fold or more in *T. maritima* in co-culture while 68 genes (25 up/43 down) were affected at this level in *P. furiosus*.

In the LPM co-culture, up-regulation of several proteases was noted, including PF0287 (subtilisin-like serine protease AprE, +3.1 fold), PF0688 (Xaa-Pro aminopeptidase AprE, +2.2 fold), PF0702 (Xaa-Pro Aminopeptidase PepP, +2.0 fold), PF0747 (Xaa-pro Aminopeptidase PepP, +2.1 fold), all of which might have been used for proteolytic degradation of extracellular proteins and peptides present in the media. The up-regulation of PF0346 (+2.1 fold) and PF1203 (+2.1 fold), both putative aldehyde-ferredoxin oxidoreductases, was also observed. AOR (PF0346) is the primary enzyme responsible for oxidizing aldehydes produced by the 2-α–keto acid oxidoreductases (47). Co-culture conditions also triggered up-regulation of PF0442 (BglB +2.0 fold), a β-glucosidase belonging glycosylhydrolase family 1 (E.C. 3.2.1.21-cellobiase, β-D-glucosidase), presumably involved in cellobiose utilization. Down-regulation of several genes associated with the synthesis of amino acids was noted in the co-culture, including: PF0935 (IlvB, -2.0 fold), PF0936 (IlvC, -4.7 fold), PF0937 (LeuA, -3.2 fold), PF0938 (LeuC, -2.8 fold), PF0939 (LeuD, -2.4 fold), PF0940 (LeuB, -2.8 fold), PF0941 (LeuA, -3.2 fold), PF0942 (IlvD, 3.1 fold), PF1054 (ThrB, -2.9 fold), PF1055 (ThrC, -2.8 fold), PF1056 (Asd, -3.4 fold), PF1678 (LeuA, -4.9 fold), PF1679 (LeuC, -5.3 fold), PF1680 (LeuD, -3.0 fold), PF1683 (ArgC, -2.5 fold), PF1684 (ArgB, -2.1 fold), PF1685 (GabT, -2.4 fold), PF1686 (ArgE, -2.3 fold); these proteins are implicated in the synthesis of amino acids, such as leucine, isoleucine, valine, arginine and aspartate. Genes associated

with the truncated TCA cycle present in this microorganism were also repressed, including: PF0201 (aconitase AcnA, -2.4 fold), PF0202 (isocitrate dehydrogenase Icd, -2.7 fold) and PF0203 (citrate synthase, GltA, -2.7 fold). In the pure culture, *P. furiosus* apparently relies, at least initially, on different carbon/energy sources as indicated by the down-regulation in the co-culture of the following transporter genes, previously implicated in the uptake and assimilation of maltose, starch, laminarin and chitin (40), including: PF1739 (Ugp, -9.3 fold), PF1742 (RfaG, -5.4 fold), PF174 (TrmB, -3.8 fold), PF1744 (MalK, -2.3 fold), PF1881 (Ssh1, -2.4 fold), PF1933 (MalK, -2.2 fold), PF1934 (AmyA, -2.6 fold), PF1936 (UgpE, -2.3 fold), PF1938 (MalE -2.5 fold). Taken together, it is apparent that in the co-culture, *P. furiosus* utilizes cellobiose primarily, but also proteins and peptides. However, in the pure culture, *P. furiosus* prefers other substrates present in the yeast extract. This may relate to the depletion by *T. maritima* of bioavailable metabolites, such as trehalose and small peptides. This, perhaps, explains why in the pure culture *P. furiosus* transcriptional machinery is devoted to the uptake and utilization of carbon sources other than cellobiose. Reasons for up-regulation of extracellular proteases in *P. furiosus* in the co-culture media were not clear. However, previous reports on *Lactococcus lactis* showed that small di-peptides and tri-peptides present in the media serve as inhibitor of the production of proteinases (40); maybe this is also the case for *P. furiosus*.

Transcriptional response of *T. maritima* in co-culture with *P. furiosus* growing in LPM at 80°C. In contrast to *P. furiosus*, only 38 genes (16 up/22 down) were differentially expressed in *T. maritima* in LPM (see Figure 1.5 and Table 1.16).

Among the up-regulated genes was an operon, TM0979-TM0983, +3.8 fold), previously associated with the response of *T. maritima* to sulfur (Conners and Kelly, unpublished data), and related to uncharacterized hypothetical proteins (*dsr* operon) associated with the oxidative sulfur metabolism in *Allochromatium vinosum* (14). A few ribosomal proteins were up-regulated: TM1483 (+2.0 fold), TM1484 (+2.0 fold), TM1491 (+2.1 fold), TM1492 (+2.1 fold), TM1498 (+2.3 fold), TM1500 (+2.3 fold), as well as DnaK (TM0373, +2.6 fold) and GroES (TM0505, +2.0 fold). Among the genes that were down-regulated in co-culture were two cold shock proteins, TM1683 (-2.5 fold), TM1874 (-2.0 fold), as well as TM0192 (-2.8 fold) , TM0266 (-3.5 fold), homologs of the regulatory protein SpoVS and the HU-DNA binding protein, respectively (49).

Growth of *P. furiosus* and *T. maritima* in each other's spent media. In addition to co-culture, the growth of each hyperthermophile in the other's spent media was also examined to determine if pure culture extracellular products impacted the transcriptomes. As noted above, a number of small ORFs encoding putative peptides were up-regulated in *P. furiosus* and *T. maritima* in co-culture, which may reflect activation of ecological strategies akin to that observed for TM0504 produced in *T. maritima*/*M. jannaschii* co-culture (30, 31). When *T. maritima* co-culture in HPM was compared with *T. maritima* pure culture in spent *P. furiosus* HPM, no significant differential expression was noted, perhaps a function of the rich medium used (see Figure 1.6). Thus, *P. furiosus* and *T. maritima* were each grown in pure culture at 80°C to > 10^7 cells/ml on LPM, following which the supernatant was recovered and inoculated with the other organism. Samples for

transcriptional analysis of each hyperthermophile were taken from cultures at approximately 10^7 cells/ml for comparison to growth in fresh media.

Transcriptional response of *P. furiosus* growing at 80°C in spent *T. maritima* LPM. A total of 108 genes (53 up/55 down) were differentially expressed in *P. furiosus* growing in spent *T. maritima* media compared to *P. furiosus* growing in fresh media (see Tables 1.2, 1.14 and Figure 1.7). As observed in the HPM co-culture experiment, up-regulation of several proteases was noted: PF0287 (subtilisin-like protease AprE, +4.9 fold), PF0747 (Xaa-pro Aminopeptidase PepP, +2.6 fold), PF1547 (FrvX +2.1 fold), a putative glutamyl-aminopeptidase (49), and PfpI (PF1719 +2.2 fold) a highly stable multimeric intracellular protease active towards basic and bulky hydrophobic amino acid residues in peptide substrates (27). The up-regulation of PF0346 (+3.3 fold) and PF1203 (+3.4 fold), both putative aldehyde-ferredoxin oxidoreductases, may indicate the generation of aldehydes produced by ketoacid oxidoreductases. The utilization of cellobiose is suggested by the up-regulation of PF0442 (BglB +2.2 fold), a β-glucosidase belonging to glycosylhydrolase family 1 (E.C. 3.2.1.21-cellobiase, β-D-Glucosidase) (13). Spent media also induced xanthine/uracil permease UraA (PF1240, +3.5 fold) and uracil phosphoribosyltransferase PF1241, +3.5 fold). It was also observed that in spent media, genes in *P. furiosus* associated with the truncated TCA cycle were down-regulated: aconitase A AcnA (PF0201, -3.2 fold), isocitrate dehydrogenase Icd (PF0202, -3.0 fold), citrate synthase GltA (PF0203, -3.9 fold). Also down-regulation of genes associated with the synthesis of amino acids such as His, Leu, Ile, Gln, Glu, Arg and Val: glutamine synthase GltB (PF0204, -2.3 fold), a cluster of genes PF0943-PF0942

(average -4.4 fold and PF1678-PF1680 (average -5.8 fold) associated with the synthesis of leucine. A biosynthetic operon associated with the synthesis of histidine PF1657-PF1662 (-2.7 fold) was also down-regulated. Interestingly, genes associated with the uptake of α-linked sugars were downregulated: ABC sugar transport ATPase MalK (PF1744, -2.1 fold), ABC sugar transport ATPase Malk (PF1933, -2.3 fold), an alpha amylase (PF1935, -3.4 fold), an ABC sugar transport permease (PF1363), and a Maltose binding protein (PF1938, -2.4 fold change). Taken together, it is possible that in pure culture, carbon and amino acids sources from yeast extract are preferred over cellobiose and other metabolites in LPM

Transcriptional response of *T. maritima* growing in spent *P. furiosus* LPM at 80°C. When grown in spent *P. furiosus* medium, 39 genes (34 up/5 down) were differentially expressed compared to growth in fresh medium. These included: ribosomal protein L28 (TM0255, -2.0 fold), a dual probe for tmRNA and a putative signal peptide (TM0504, -5.4 fold) (see Chapter 3), a crossover junction endonuclease (TM0575, -2.1 fold), a hypothetical protein (TM1125, -2.1 fold), rRNA 5S (-3.7 fold), proteins involved in biosynthesis of leucine and threonine, threonine synthase (TM0546, +2.3 fold), homoserine kinase (TM0545, +2.4 fold), acetolactate synthase IlvH (TM0549, +2.5 fold), ketol acid reductoisomerase IlvC (TM0550, +2.7 fold), dihydroxyacid dehydratase IlvD (TM0551, +2.5 fold), isopropylmalate homocitrate synthase LeuA (TM0552, +2.4 fold), isopropylmalate homocitrate synthase LeuA (TM0553, +2.8 fold), 3-isopropylmalate dehydratase LeuC (TM0554, +2.8 fold), 3-isopropylmalate dehydratase LeuD (TM0555, +3.5 fold), isocitrate isopropylmalate dehydrogenase LeuB (TM0556, +3.1 fold). In addition, a methyl-accepting chemotaxis protein, Tar (TM1146, +2.8 fold), and a

response regulator (TM1147, +4.0 fold), which according to the information contained in the database of conserved domains (49), are associated with signal transduction and the response to repellent or attractant molecules, were induced.

TM1300-1338 locus. Of particular interest in the set of up-regulated ORFs in *T. maritima* growing on *P. furiosus* spent LPM was the locus TM1300-TM1338 (see Figure 1.8) whose differential expression had not been noted previously in transcriptional response experiments with *T. maritima* (see Table 1.5). Bioinformatic analysis of the TM1300-TM1338 locus (see Figure 1.9) revealed that it contained several small hypothetical proteins of less than 80 amino acids (TM1300, TM1315, TM1316, TM1323, TM 1333), components of putative transporters (TM1302-1304, TM1318-1319, TM1326-1328, TM1336), and putative Fe-S-oxidoreductases (TM1301, TM1317, TM1324-1325, and TM1334-1335). The oxidoreductases have been annotated as AstB/ChuR-related proteins, belonging to the S-adenosyl methionine (SAM) radical superfamily (39, 51, 72). These proteins are characterized by a –CXXXCXXC- motif associated with the formation of 4Fe-4S clusters, and a double glycine motif implicated in the binding of SAM (72). In these oxidoreductases, only three of the 4 Fe atoms are coordinated by the protein, causing the extreme lability of the cluster and its inactivation under mild oxidizing conditions (51). Current thinking is that these enzymes are involved in the reduction of iron sulfur clusters, mediated by a flavodoxin. This then initiates the cleavage of SAM, resulting in the release of methionine and a highly reactive 5'deoxyadenosine radical, important for the catalysis of chemically challenging reactions (39).

SAM radical proteins catalyze a diverse range of reactions, such as unusual methylations, isomerizations, sulfur insertions, anaerobic oxidations and ring formations (39, 72). Along these lines, SAM radical superfamily proteins have been associated with the formation of unusual α-carbon linkages, for instance, in the quinohemoprotein amine dehydrogenase of *Paracoccus denitrificans* (15). These linkages are formed between thiol (cysteine) groups and tryptophan, glutamic acid or aspartic acid, mediated by an accessory protein (CTQ-ORF2, gi17402569, accession BAB78727) via a still uncharacterized mechanism. (15). Similar α-carbon cross-links have also been observed in a bacteriocin produced by *Bacillus subtilis*, subtilosin A (77). In this case, another SAM radical family protein, AlbA, is responsible for post-translational modification of this bacteriocin such that a cyclic peptide is formed involving α-carbon cross links between Cys residues and amino acids. AlbA belongs to the COG0535 Fe-S-oxidoreductases, which corresponds also to the same functional assignment of TM1317. The overall sequence similarity between TM1317 and AlbA (20% identity, 35% similarity) and CTQ-ORF2 (23% identity, 41% similarity) is significant in a sense that all functional domains are conserved between these proteins.

Comparison of the biosynthetic gene cluster of Subtilosin A (see Figure 1.9 and Table 1.6) and ORFs within the TM1300-TM1338 region (see Table 1.5) revealed several similar features: components of putative transporters, radical SAM family proteins and small ORFs. No sequence similarity was found between Subtilosin A and any genes in the TM1300-TM1338 locus. However, the putative peptide encoded in TM1316, which was up-regulated 19-fold in the spent media, was found previously in tryptic digests of concentrated *T. maritima* culture supernatants from growth on defined media (RDM) (68)

indicated the presence of a portion of TM1316 (PVVPNDYLNPGKG) (see Figure 1.10) (Johnson and Kelly, unpublished data), indicating that TM1316 is an extracelllular product. The nascent forms of Subtilosin A and TM1316 are of similar size and contain the same number and approximate spacing of cysteine residues. Further analysis showed almost identical positioning of 7 amino acids, including cysteines and 4 other conserved amino acids (see Figure 1.10). The sequence patterns [C]-X-X-[C]-X-X-[G]-[A]-X-[C]-X-X-X-[G]-[P] and [P]-[G]-X-X-[C]-X-X-[A]-[G]-X-X-[C]-X-X-[C], and variations of them of different spacing between glycine and alanine and the flanking cysteines, were used to search homologous domains to Subtilosin A and TM1316, respectively, in public data bases using "MyHits" (63). Thus, so far these domains appear to be unique.

Another interesting similarity between these two peptides emerges from the studies on the mechanism of membrane disruption of subtilosin A. The mechanism of action of Subtilosin A involves membrane disruption by partially burying itself in the lipid bi-layer, inducing a conformational change in the lipid head group. At high peptide concentrations, this results eventually in membrane permeabilization through a mechanism that is apparently independent of pore formation (33, 77). Based on fluorescence spectroscopy and solid state NMR, the residue W34 (possibly equivalent to the residue W29 in the TM1316 molecule) is buried in the phospholipid bilayer, while the positively charged K2 residue (K3 in TM1316) interacts with phospholipid heads (77). In Subtilosin A, K2 is flanked by asparagine (N1), while in TM1316, K3 is flanked by glutamine Q2. On the other hand, W34 in Subtilosin A is adjacent to G35, whereas in TM1316, W29 is adjacent to C30 and G31. It is not clear to what extent C30 might be involved in the interaction of TM1316, as a putative lipid bi-layer intercalating peptide,

but this issue merits further investigation. Physicochemical properties predicted by ProtParam (21) for TM1316 and Subtilosin A suggested important differences (See Table 1.7). For instance, Subtilosin A is an anionic peptide with an aliphatic value of 86.94 whereas TM1316 is cationic with an aliphatic value of 56.45; for TM1316, these resemble the physicochemical properties predicted by ProtParam for two pore-forming, cross-linked bacteriocins: Nisin A and Lacticin 481 (8).

The reasons behind the induction of genes in the TM1300-TM1338 locus for *T. maritima* growing on *P. furiosus* spent media remains unclear. This locus was not up-regulated in the co-culture nor during other conditions examined thus far. Whether, in fact, TM1316 or other peptides/proteins within the locus are triggered as an antagonistic or competitive response remains to be seen. This may have been a factor given the induction of the biosynthetic genes for leucine, perhaps arising from a severe nutritional limitation, and TM1146-TM1147, which encode signal transduction/chemotaxis genes. Furthermore, the up-regulation of ORFs encoding a number of putative small peptides in *T. maritima* and *P. furiosus* under various growth conditions (see Table 1.4) is also intriguing and may reflect mechanisms by which these hyperthermophiles respond to environmental stimuli. Along these lines, two putative small ORFs differentially expressed in the co-culture, PF2058 (+2.3 fold) and PF2057 (+2.5 fold), are located in a gene neighborhood in the *P. furiosus* genome that includes an ABC transporter component (PF2061, +2.0 fold) in addition to a SAM radical superfamily proteins, PF2060 (no change) and PF2064 (no change) which are the most similar in the genome of *P. furiosus* to AstB/ChuR proteins (see Figure 1.11). Interestingly this gene neighborhood includes putative N-terminal aminopeptidases (PF2059, PF2063 and

PF2065) none of which were differentially transcribed. PF2057 and PF2058, according to ProtParam (21), encode putative peptides which are soluble and stable with pIs of 9.35 and 5.81 and an aliphatic index of 111.25 and 127.58, respectively. Given the situation in *T. maritima* with TM1300-TM1338, this locus in *P. furiosus* merits further study.

Summary. In this study, it was shown that cDNA microarray technology can be used to examine simultaneous trancriptional profiles of phylogenetically distant microbial species. This approach was applied to the study of a co-culture of two hyperthemorphilic microorganisms, a bacterium *T. maritima* and an archaeon *P. furiosus.* Insights into the regulatory controls associated with the microbial interaction of these two heterotrophs were collected and exemplify the profound effects of media composition and cell density during sampling points. Differential transcription of small protein of unknown function was observed. Among them, TM1316, a small 31aa peptide, was found to be co-transcribed with adjacent genes, including components of putative transporters, and radical SAM superfamily proteins. Similar genomic organization to the *B. subtilis sbo-alb* operon and resemblance between the *sboA* structural gene of the bacteriocin Subtilosin A (87) and TM1316 suggests the response in *T. maritima* of a putative signaling (or bacteriocin) molecule upon exposure to *P. furiosus* spent media. The present approach contributes to the understanding of microbial interactions by providing insights on otherwise inaccessible responses of microbial microorganisms to their ecological interactions. Beyond this study of bacterial and archaeal species, this strategy can be readily adapted and implemented upon optimization of probe design to the study of microbial interactions of non-hyperthermophilic species or even more closely-related species from a phylogenetic perspective.

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Table 1.1. Growth of *P. furiosus* and *T. maritima* in LPM and HPM

Organism	Condition	Medium	Maximum cell density	Density at harvest	
				High cell density	Low cell density
P. furiosus	Pure	HPM	3×10^8	9.9×10^7	2.5×10^7
T. maritima	Pure	HPM	3.2×10^8	1.2×10^8	1.6×10^7
P. furiosus	Co-culture	HPM	1.9×10^8	9.7×10^7	2.8×10^7
T. maritima	Co-culture	HPM	4.0×10^8	1.0×10^8	2.4×10^7
T. maritima	Pure	HPM (Pfu spent)	8.9×10^8	1.1×10^8	n.d.
T. maritima	Pure	LPM	2.0×10^8	n.d.	2.7×10^7
P. furiosus	Pure	LPM	3.5×10^8	n.d.	1.5×10^7
T. maritima	Co-culture	LPM	2.3×10^8	n.d.	2.0×10^7
P. furiosus	Co-culture	LPM	2.2×10^8	n.d.	3.6×10^7
T. maritima	Pure	LPM (Pfu spent)	2.0×10^8	n.d.	1.3×10^7
P. furiosus	Pure	LPM (Tma spent)	4.1×10^8	n.d.	1.5×10^7

n.d. - refers to conditions not tested

Table 1.2. Summary of differentially transcribed ORFs* in co-culture and spent media growth experiments

Condition		Up-regulated	Down-regulated	Total
HPM				
Comparison	**Harvest***			
Co-culture vs. Pfu pure culture	High cell density	**212**	**239**	**451**
Co-culture vs. Tma pure culture	High cell density	**20**	**36**	**56**
Co-culture vs. Pfu pure culture	Low cell density	**36**	**28**	**64**
Co-culture vs. Tma pure culture	Low cell density	**214**	**120**	**334**
LPM				
Comparison	**Harvest***			
Co-culture vs Pfu pure culture	Low cell density	**25**	**43**	**68**
Co-culture vs Tma pure culture	Low cell density	**16**	**22**	**38**
Spent Media (HPM)				
Comparison	**Harvest***			
Co-culture vs. Tma pure culture in spent Pfu	High cell density	**0**	**0**	**0**
Spent Media (LPM)				
Comparison	**Harvest***			
Pfu in spent Tma vs. Pfu pure culture	Low cell density	**53**	**55**	**108**
Co-culture vs. Pfu pure culture in spent Tma	Low cell density	**23**	**11**	**34**
Tma in spent Pfu vs. Tma pure culture	Low cell density	**34**	**5**	**39**
Co-culture vs. Tma pure culture in spent Pfu	Low cell density	**11**	**43**	**54**

HPM – High peptide media (31)

LPM- Low peptide media (65)

*"low cell density" - harvested between 1 x 10^7-2.8 x 10^7cells/ml

*"high cell density" – harvested between 9.9 x 10^7-1.2 x 10^8cells/ml

(*) Differential transcription was defined as ORFs changing ≥ 2-fold.

Table 1.3. Genes up-regulated in *T. maritima* at low cell density co-culture in HPM							
Gene ID	**Gene name**	**Fold Change**	**-Log(10)pval**	**Size**	**Strand**	**AA**	**COG_function**
TM0429	Tar	3.1	15.8	1971	-	657	Methyl-accepting chemotaxis protein
TM0463	LspA	2.9	14.4	561	-	187	Lipoprotein signal peptidase
TM0627	RfaG	2.3	8.3	1182	-	394	Glycosyltransferase
TM0630	WcaG	2.3	10.7	1041	-	347	Nucleoside-diphosphate-sugar epimerases
TM0631	RfaG	2.1	13.8	1305	-	435	Glycosyltransferase
TM0700	CheY	2.2	12.1	363	-	121	FOG: CheY-like receiver
TM0701	CheW	2.7	12.6	456	-	152	Chemotaxis signal transduction protein
TM0702	CheA	8.1	6.0	2016	-	672	Chemotaxis protein histidine kinase
TM0744	RfaG	2.3	13.8	1221	-	407	Glycosyltransferase
TM0842	CheY	3.1	17.0	762	+	254	FOG: CheY-like receiver
TM0872	-	2.2	12.6	900	-	300	Predicted S-adenosylmethionine-dependent methyltransferase
TM0903	CheD	2.5	13.8	474	-	158	Chemotaxis protein;
TM0904	CheC	3.1	15.4	618	-	206	Chemotaxis protein CheC
TM1360	CheY	2.7	18.5	351	+	117	FOG: CheY-like receiver
TM1442	SpoI	8.1	14.2	333	+	111	Anti-anti-sigma regulatory factor
TM1654	AtoS	2.5	13.9	1239	-	413	FOG: PAS/PAC domain

Table 1.4. Differential transcription of small unknown reading frames in co-cultures of *T. maritima* and *P. furiosus* grown in HPM

Gene	Fold Change Co-culture vs Pure Culture	Size (aa)	Domain/Features
PF0066	1.9	44	Transmembrane domain; exclusive to *Thermococcales*
PF0228	1.9	67	Transmembrane domains; exclusive to *Pyrococcus sp.*
PF0423	1.9	50	
PF0433	2.1	51	Methyl-accepting domain; exclusive to *Pyrococcus sp*.
PF0961	2.2	72	Signal peptide and transmembrane domain
PF1319	2.1	37	Exclusive to *Pyrococcus sp*.
PF1527	2	133	Signal peptide
PF1681	2	55	LysW homolog exclusive to archaea and *Lactobacillus plantarum*
PF2057	2.4	56	Exclusive to archaea
PF2058	2.3	65	Exclusive to archaea
TM0147	1.9	111	Conserved bacterial protein; DUF143 domain
TM0170	2.1	74	Exclusive to *Thermotogales*
TM0210	2	70	Exclusive to *Thermotogales*
TM0687	3.3	119	Conserved bacterial protein; DUF149 domain
TM0693	4.7	91	Exclusive to *Thermotogales*
TM1381	3.7	130	Exclusive to *Thermotogales*
TM1625	1.9	134	Exclusive to *Thermotogales;* UvR domain
TM1630	2	101	Exclusive to *Thermotogales*
TM1764	2.3	122	Exclusive to *Thermotogales*; DUF322 domain
TM1871	2.3	92	Exclusive to *Thermotogales*

Table 1.5. Features of genes associated with the TM1300-TM1338 cluster					
Gene	**Length (aa)**	**Signal Peptide**	**COG**	**Product**	**Domain /Features***
TM1300	80	25		Hypothetical protein	Highly similar to TM1315 but extra 22aa
TM1301	443	None	COG0641	astB/chuR-related protein	Radical Sam/AslB
TM1302	259	None	COG1131	ABC transporter, ATP-binding protein	Similar to BcrA (bacitracin resistance gene)
TM1303	254	None	COG0842	ABC transporter permease	ABC permease MDR transport system
TM1304	272	None	COG0842	ABC transporter permease	MDR transport system ABC type2
TM1305	44	28		Hypothetical protein	No homolog in Genbank
TM1306	145	37	COG0842	Conserved hypothetical	ABC-type multidrug transport system, permease
TM1307	128	None		Cypothetical protein/transmembrane	Has a single transmembrane region
TM1308	32	None		Hypothetical protein/transmembrane	Has a single transmembrane region
TM1309	69	18		Hypothetical protein/transmembrane	Signal peptide and transmembrane region
TM1310	581	None	COG1132	ABC transporter, ATPase subunit	ABC-type multidrug transport system, ATPase
TM1311	71	None		Hypothetical protein	UPF0150 uncharacterized protein family
TM1312	78	None	COG1724	Conserved hypothetical	Predicted periplasmic or secreted lipoprotein
TM1313	76	None	COG1598	Conserved hypothetical	Uncharacterized conserved protein
TM1314	536	None		Conserved hypothetical	HTH-DNA binding motif Xre Family
TM1315	58	25		Hypothetical protein	Signal peptide
TM1316	32	None		Hypothetical protein	No homologs in Genbank.
TM1317	464	None	COG0535	astB/chuR-related prote	Predicted Fe-S oxidoreductases Radical _SAM

Table 1.5. (cont.) Features of genes associated with the TM1300-TM1338 cluster					
Gene	**Length (aa)**	**Signal Peptide**	**COG**	**Product**	**Domain Features**
TM1318/ TM1319	565	None	COG1132	ABC transporter, ATP-binding protein	ABC-type multidrug transport system, ATPase
TM1320	228	None		Hypothetical protein/frameshift	
TM1321	73	None	COG1598	Conserved hypothetical	Belongs to family UPF0150
TM1322	537	None		Conserved hypothetical	96% identity with TM1332, 50% identity to TM1314
TM1323	61	17		Hypothetical protein	Signal peptide present
TM1324	442	None	COG0641	astB/chuR-related protein	SAM, AslB, Elp3
TM1325	454	None	COG0641	astB/chuR-related protein	SAM, AslB, Elp3
TM1326	286		COG0842	Conserved ABC multidrug transporter	ABC type 2 permease
TM1327	285	None	COG4555	ABC-type Na+ transport system, ATPase component	ABC/MDR
TM1328	572	None	COG1132	ABC-type multidrug transport system, ATPase	MdlB-membrane-ATPase
TM1329	80	None		Hypothetical	Has a RRRR motif present
TM1330	112	None	COG1396	Conserved hypothetical	HTH-DNA binding motif Xre Family
TM1331	144	None		Hypothetical protein	No homologs in Genbank
TM1332	523	None		Conserved hypothetical	96% identity with TM1322, 50% identity to TM1314
TM1333	61	17		Hypothetical protein	Identical to TM1323

Table 1.5. (cont.) Features of genes associated with the TM1300-TM1338 cluster					
TM1334	595	None	COG1032	Hypothetical protein	Elp3/Radical SAM protein
TM1335	643	None		Hypothetical protein	
TM1336	391	None	COG0477	TetV homolog	DUF894 Permease MFS-1
TM1337	259	22		Hypothetical protein	
TM1338	45	None		Hypothetical protein	Cationic peptide pI 10.22
Description of Domains and features corresponds to a contribution from different bioinformatic tools such as SMART, CDD and COG database (43, 49, 76), protein analysis using Protparam (21), and conventional BLAST searches (56)					

Table 1.6. Features of genes associated with the *sbo-alb* operon

Gene	Length (aa)	Signal peptide	COG	Annotation	Domain/Feature
sboA	43	None		Bacteriocin	α-C Crosslinks
sboX	50	None		Unknown function	
albA	448	None	COG0535	Modifying Enzyme	Radical Sam/ Elp3
albB	53	25		hypothethical protein	Single transmembrane region
albC	239	None	COG1131	ABC Transporter ATPase	ABC /ATPase subunit domain
albD	436	None		Integral transmembrane	10 transmembrane regions
albE	386	None	COG0612	Peptidase M16 family	Peptidase M16 (N-domain)
albF	427	None	COG0612	Peptidase M16 family	Peptidase M16 domain
albG	233	None		Integral transmembrane	5 transmembrane regions

Description of Domains and features corresponds to a contribution from different bioinformatic tools such as SMART, CDD and COG database (43, 49, 76), protein analysis using Protparam (21), and conventional BLAST searches (56)

Table 1.7. Selected physico-chemical parameters of TM1315, TM1316, and previously characterized bacteriocins

	NISIN A	Lacticin 481	Subtilosin A	TM1316	TM1315-without signal peptide
AA	57	27	36	31	55
MW	5962.9	2973.3	3555.5	3177.7	6181.9
pI	8.99	6.99	3.92	8.61	9.3
Asp+Glu[(1)]	3	1	4	1	7
Arg+Lys[(2)]	7	1	1	3	9
Estimated half life[(3)]	>10h	3 min	>10	>10	>10
Instability index [(4)]	35.11 (stable)	26.31 (stable)	24.31 (stable)	40.75 (unstable)[(7)]	30.71 (stable)
Aliphatic Index [(5)]	68.42	50.37	86.94	56.45	90.36
Grand Average Index[(6)]	-0.005	0.052	0.575	-0.123	-0.735

[(1)] = Negatively charged residues; [(2)] = Positively charged residues; [(3)] = "ProtParam" estimates the half-life by looking at the N-terminal amino acid of the sequence under investigation (21). The half-life is a prediction of the time it takes for half of the amount of protein in a cell to disappear after its synthesis in consideration to the N-terminal amino acid rule (11, 78). [(4)] = The "instability index" provides an estimate of the intrinsic stability of a protein *in vivo*. A value of 40 or above is usually associated with unstable proteins (26, 67). [(5)] = The "aliphatic index" of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). As a measurement of the hydrophobicity of a protein, high values have been observed in bacteriocins that targets bacterial membranes (77). [(6)] = The "Grand Average index" is an alternative measurement of the hydrophobicity of a protein, values above 0 are hydrophobic in character (38). [(7)] = Note that the value assigned to TM1316 (40.75) is slightly above the limit associated to stable proteins.

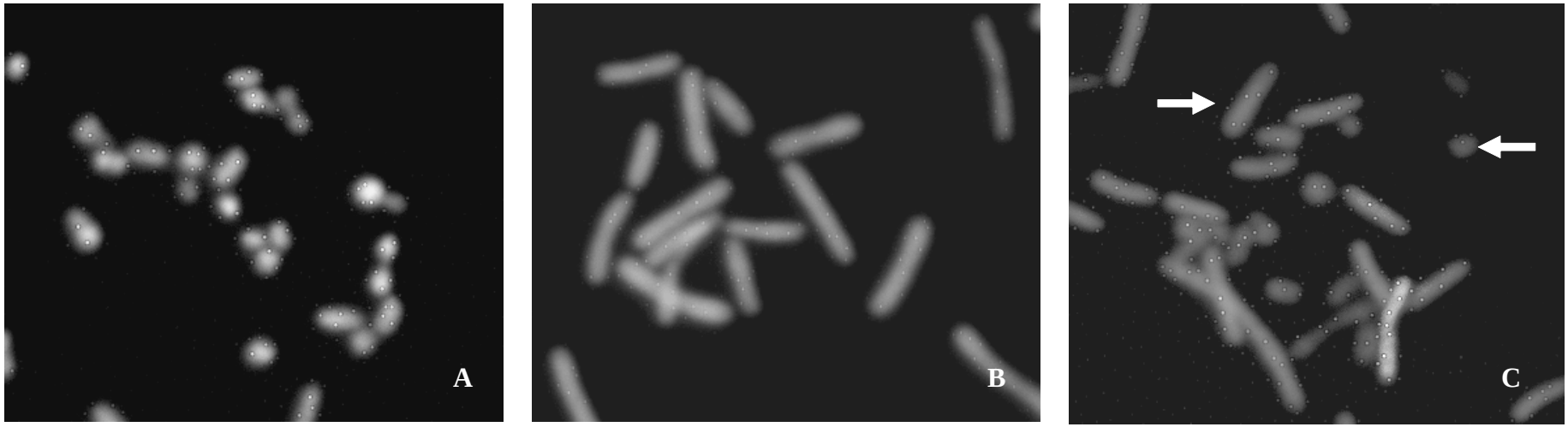
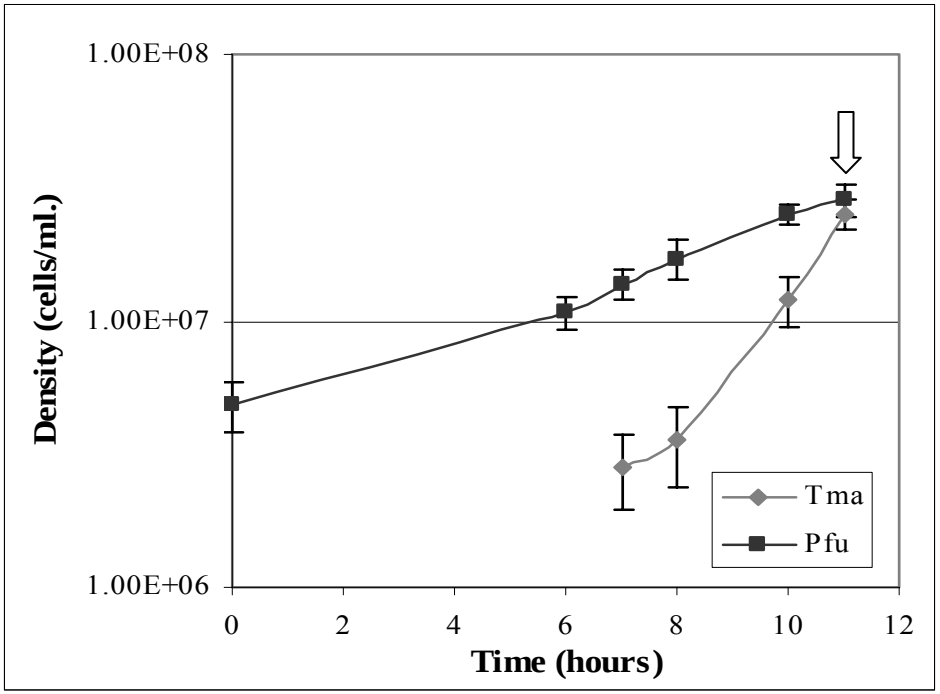
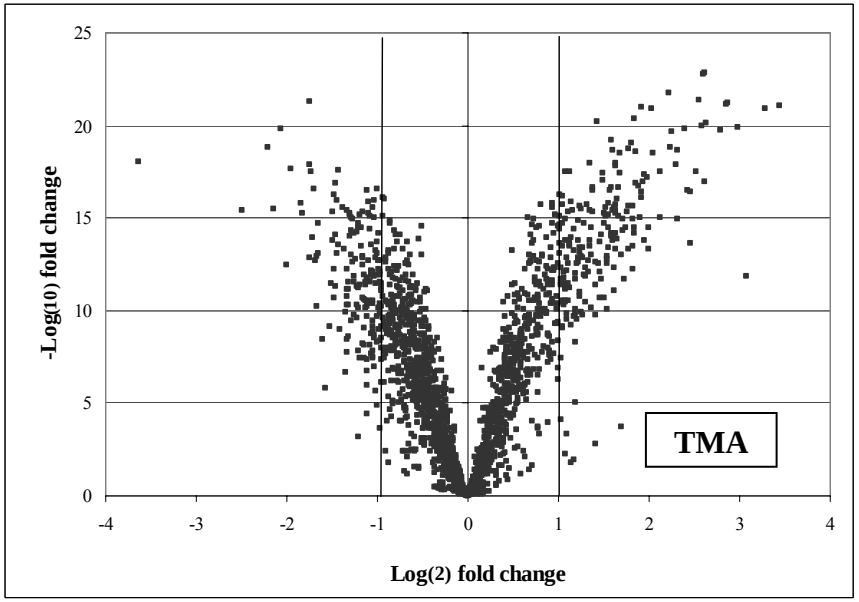


FIGURE 1.1. Typical cell morphologies for the hyperthermophilic microorganisms *T. maritima* and *P. furiosus*. Epifluorescence microscopy of glutaraldehyde-fixed cells stained with acridine orange was routinely used for cell density enumeration. Cell morphologies of *P. furiosus* (A) and *T. maritima* (B) made possible the differential estimation of cell densities when growing in co-culture (C).





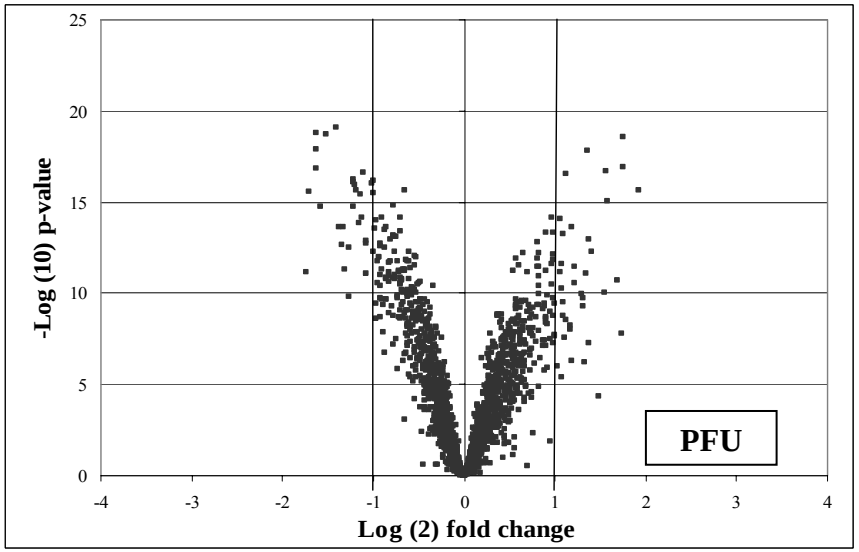
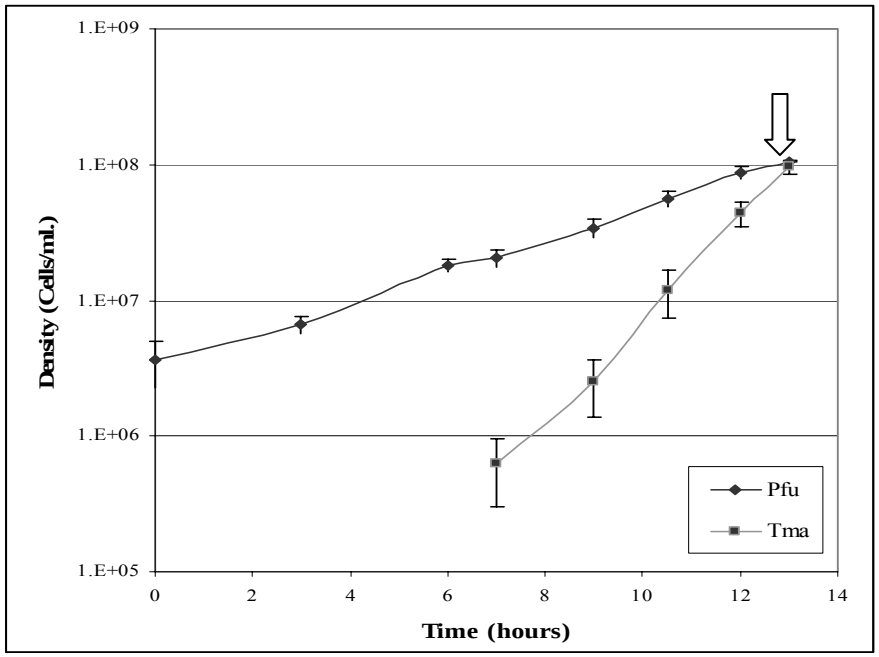
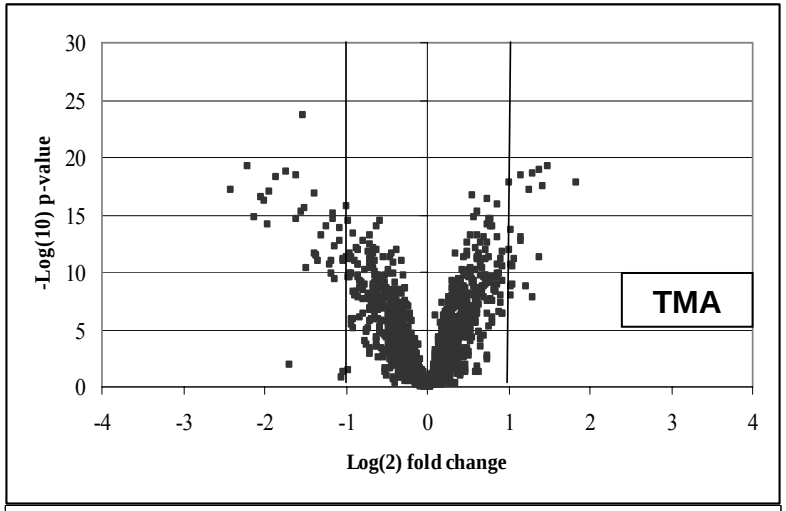


FIGURE 1.2. Co-culture in HPM – sample at low cell density: (Top) Co-culture of *T. maritima* and *P. furiosus* in HPM at 80°C. Samples for transcriptional analysis were taken when cell densities of each microorganism reached > 10^7 cells/ml. (Right) Volcano plots showing $\log_2$ fold change (horizontal axis) and $-\log_{10}$ (p-value) for comparisons between transcriptome of each microorganism in pure and co-culture. For *P. furiosus* 64 ORFs were differentially expressed 2-fold or more (36 up/28 down) in co-culture whereas for *T. maritima* 334 ORFs were affected at this level (214 up/120 down). An arrow indicates the point of harvest for RNA extraction





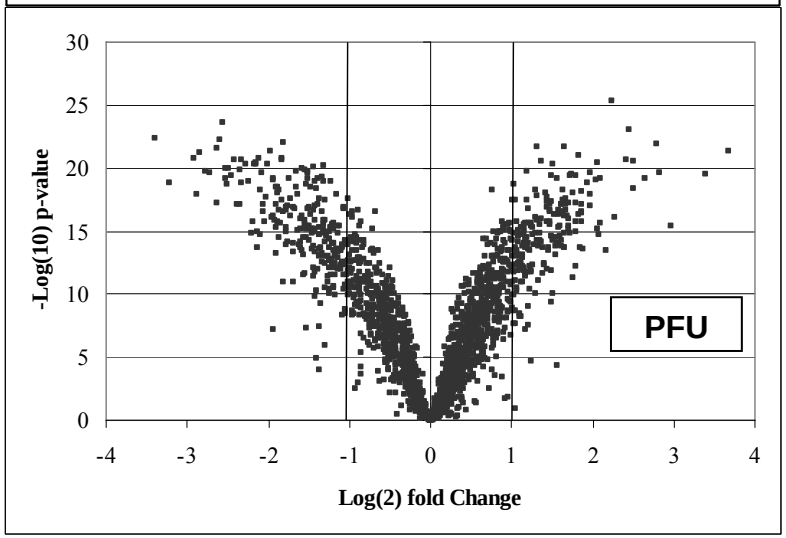
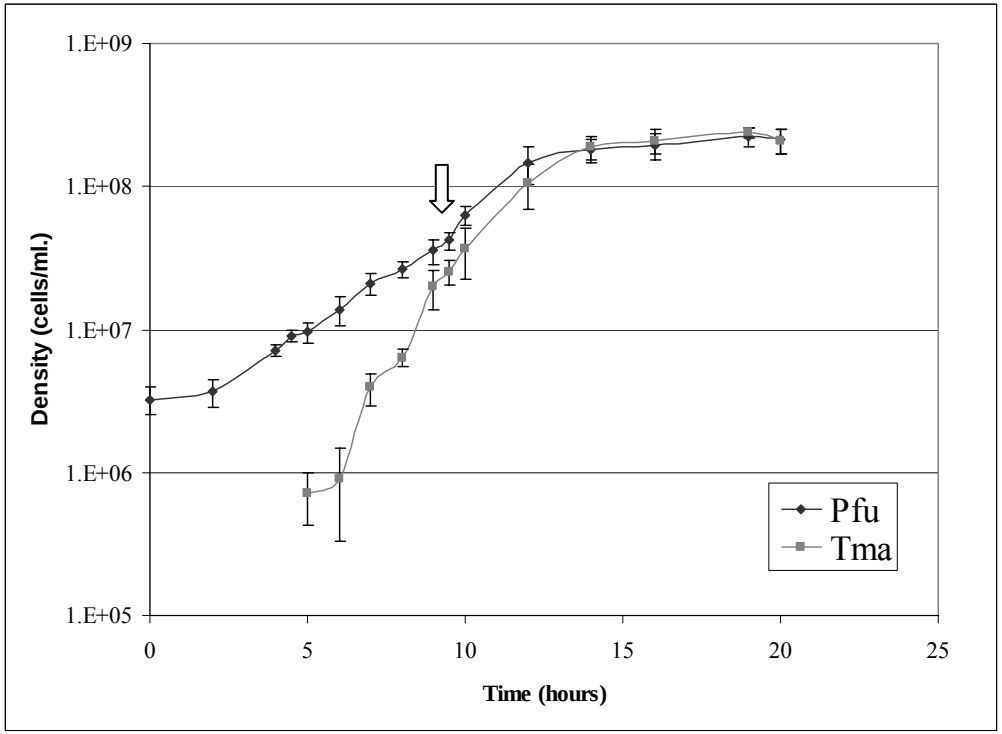
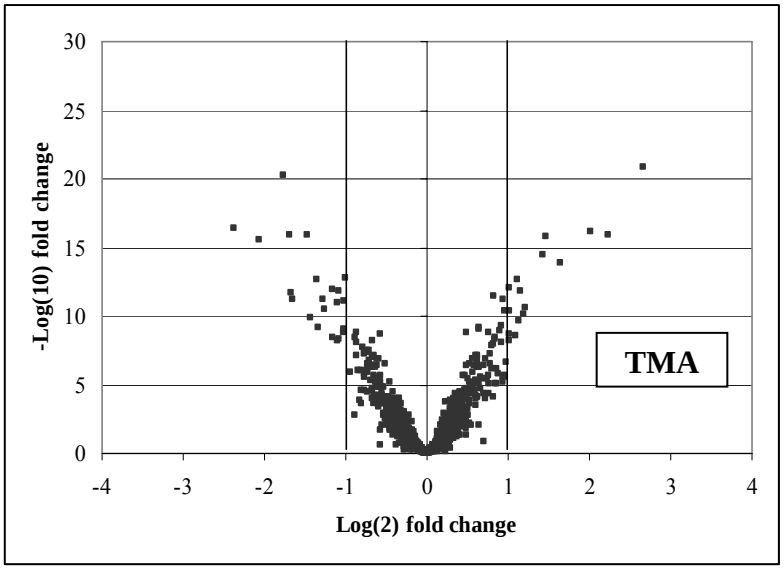


FIGURE 1.3. Co-culture in HPM – sample at high cell density: (Top). Co-culture of *T. maritima* and *P. furiosus* in HPM at 80°C. Samples for transcriptional analysis were taken when cell densities of each microorganism reached > 10^8 cells/ml. (Right) Volcano plots showing $\log_2$ fold change (horizontal axis) and $-\log_{10}$ (p-value) for comparisons between transcriptome of each microorganism in pure and co-culture. For *P. furiosus* 451 ORFs were differentially expressed 2-fold or more (212 up/239 down) in co-culture whereas for *T. maritima* 56 ORFs were affected at this level (20 up/36 down). An arrow indicates the point of harvest for RNA extraction





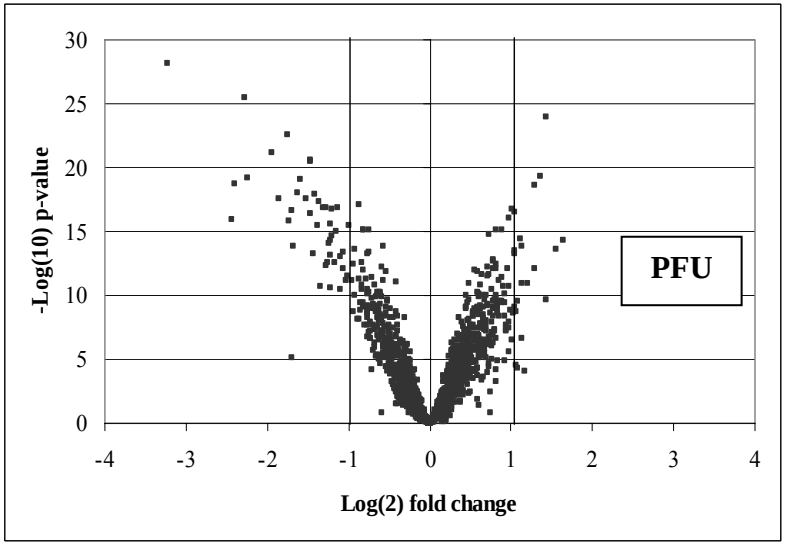
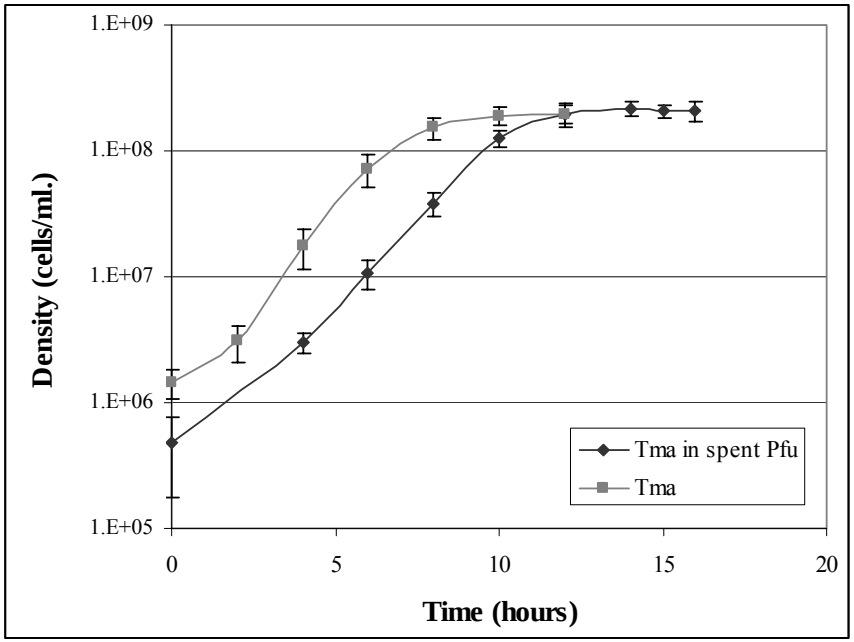


FIGURE 1.4. Co-culture in LPM – sample at low cell density: (Top) Co-culture of *T. maritima* and *P. furiosus* in LPM at 80°C. Samples for transcriptional analysis were taken when cell densities of each microorganism reached > 10^7 cells/ml. (Right) Volcano plots showing $\log_2$ fold change (horizontal axis) and $-\log_{10}$ p-value for comparisons between the transcriptome of each microorganism in pure and co-culture. For *P. furiosus* in co-culture 67 ORFs were differentially expressed 2-fold or more (24 up/43 down) while for *T. maritima* 38 ORFs were affected at this level (16 up/22 down). An arrow indicates the point of harvest for RNA extraction.



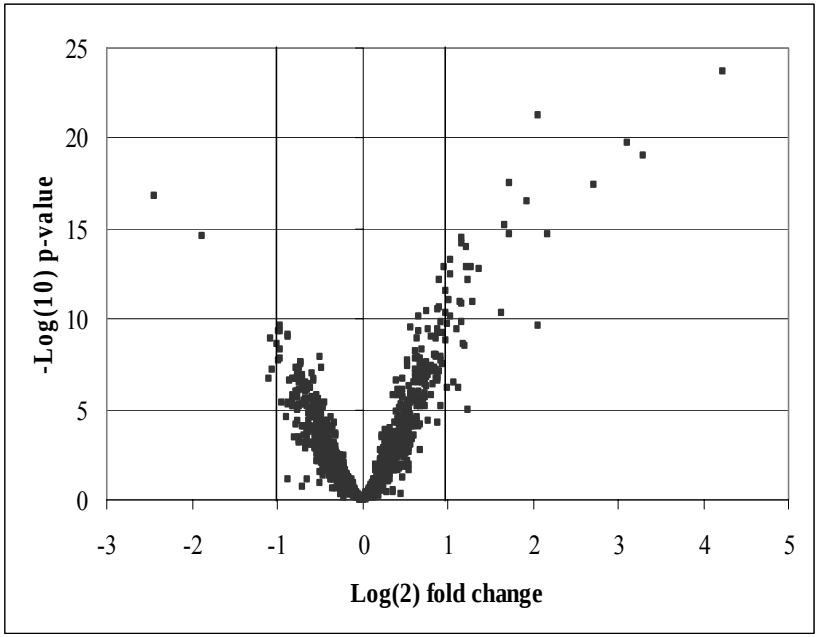


FIGURE 1.5. Growth and differential transcription of *T. maritima* in *P. furiosus* spent LPM: (Left) Growth of *T. maritima* at 80°C in spent *P. furiosus* medium and in pure culture. Samples for transcriptional analysis were taken when cell densities of each microorganism reached $>10^7$ cells/ml. (Right) Volcano plot showing $\log_2$ fold change (horizontal axis) and $-\log_{10}$ p-value for comparisons between the transcriptional profiles of *T. maritima* in spent *P. furiosus* media and in pure culture; 39 ORFs that were differentially transcribed (34 up/5 down).

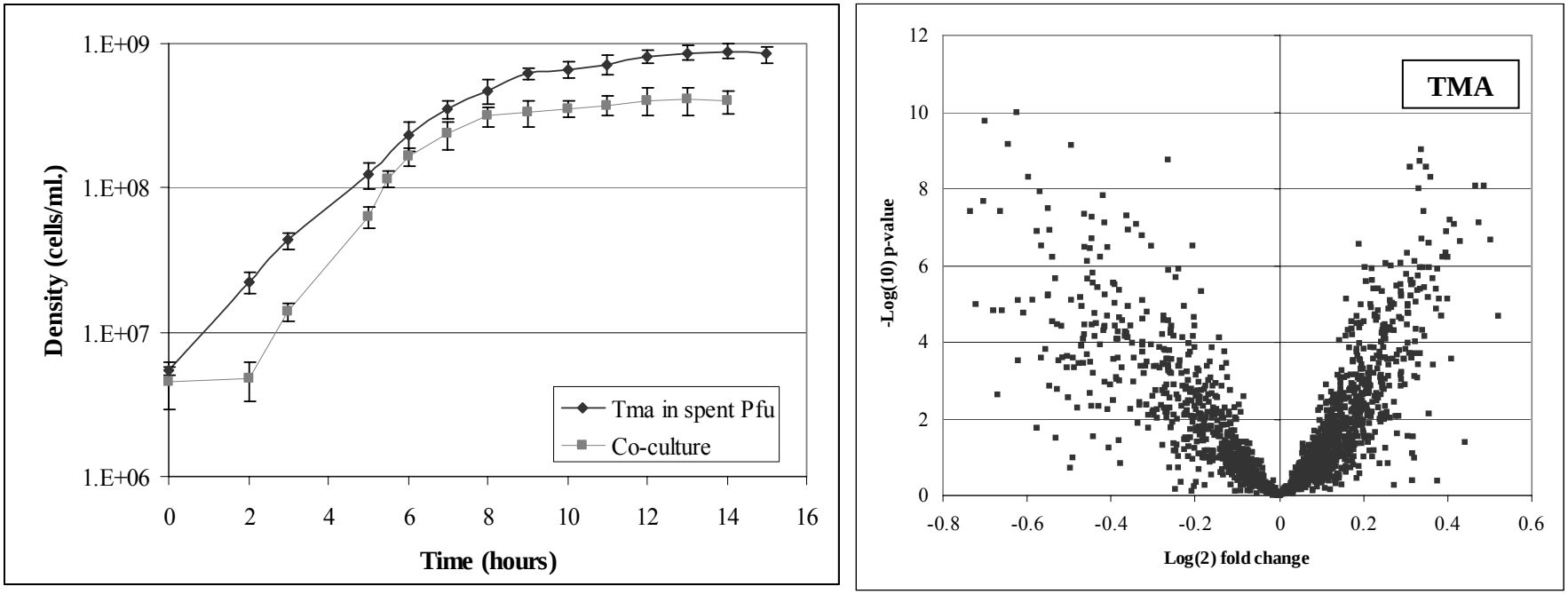


FIGURE 1.6. Growth and differential transcription of *T. maritima* growing in co-culture and in *P. furiosus* spent HPM: (Left) Growth of *T. maritima* in HPM at 80°C in spent *P. furiosus* media and in co-culture. Samples for transcriptional analysis were taken when cell densities of each microorganism reached > 10^8 cells/ml. (Right) Volcano plot showing $\log_2$ fold change (horizontal axis) and $-\log_{10}$ p-value for comparisons between the transcriptome of *T. maritima* growing in spent *P. furiosus* media and in co-culture. No gene showed more than 2-fold differential transcription.

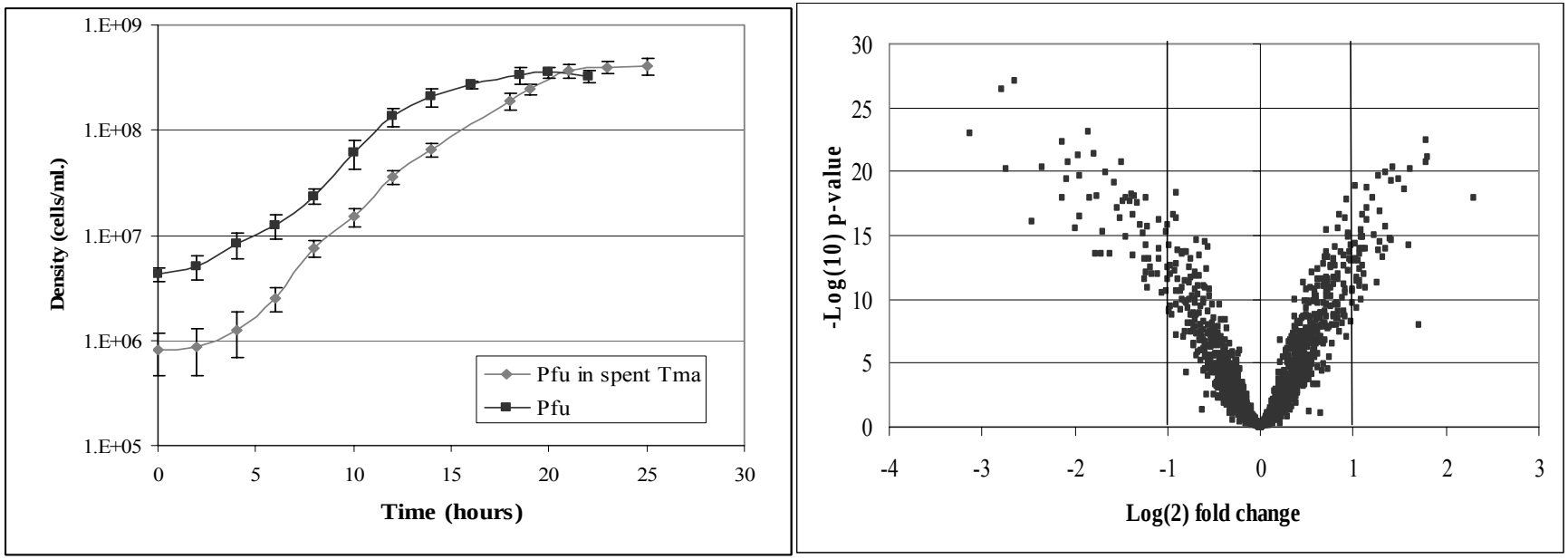


FIGURE 1.7. Growth and differential transcription of *P. furiosus* growing in *T. maritima* spent media: (Left) Growth of *P. furiosus* at 80 °C in spent *T. maritima* LPM and in pure culture. Samples for transcriptional analysis were taken when cell densities of each microorganism reached > 10^7 cells/ml. (Right) Volcano plot showing $\log_2$ fold change (horizontal axis) and $-\log_{10}$ p-value for comparisons between transcriptional profiles of *P. furiosus* growing on spent *T. maritima* and in pure culture. This resulted in 108 ORFs that were differentially transcribed (53 up/55 down)

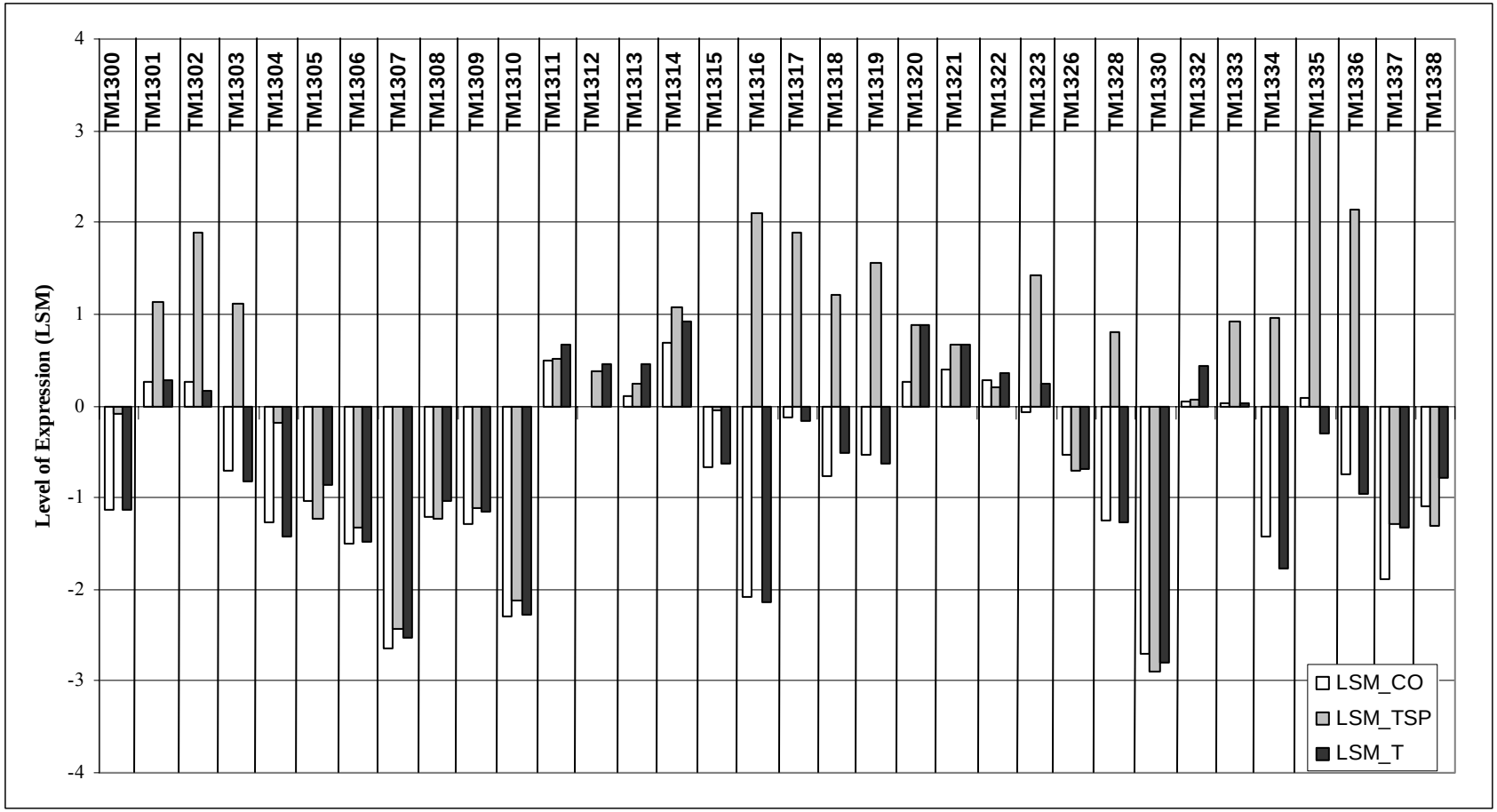


FIGURE 1.8. Transcriptional profiles of select genes showing differential expression of ORFs in the TM1300-TM1338 locus in *T. maritim*a growing on *P. furiosus* spent LPM. This experiment was performed at 80°C without any additional supplementation to the *P. furiosus* spent media. Key: CO= Co-culture condition *T. maritima* and *P. furiosus* harvested at a cell density of 10^7cells/ml. TSP = *T. maritima* grown on *P. furiosus* spent LPM. T= *T. maritima* grown in pure culture in LPM. Probes for genes TM1324, TM1325, TM1327, TM1329 and TM1331 were not included on microarray platform.

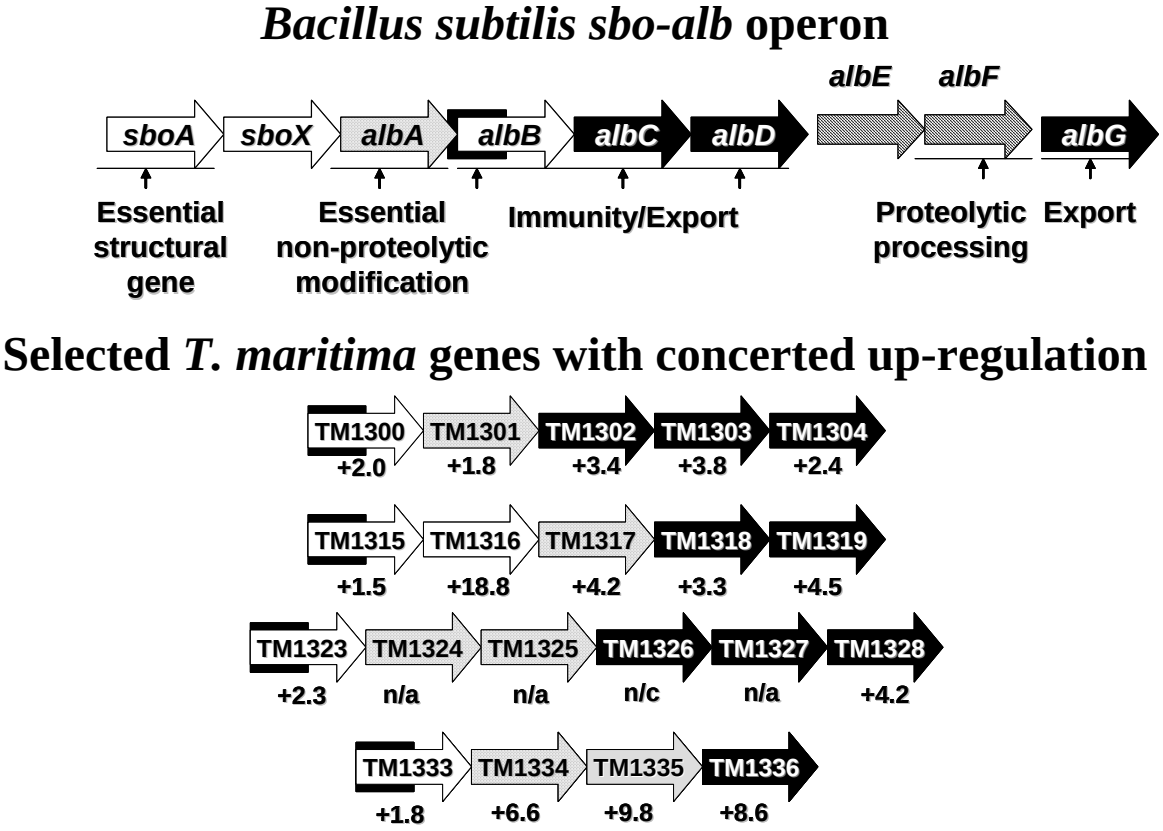


FIGURE 1.9. Comparison between the sbo-alb cluster assocaited with the production of subtilosin A in *Bacillus subtilis* and selected *T. maritima* genes in TM1300-TM1338 locus: The *sbo-alb* operon schematic was adapted from Zheng *et al.* 2002 following their suggested functional assignment. All the ORFs are coded according to their structural features and domains: Genes that are represented as white arrow (⇨) corresponds to small ORF (<80aa). Genes represented as dotted arrow (⇨) are members of the SAM-Radical superfamily. Show as grey arrows (⇨) are genes of unknown function. (⇨) refers to genes associated with the proteolytic processing of SboA (Subtilosin A).ORFs represented as black arrows (⇨) are putative component of ABC transporters. There is significant homology (91% identity at the a.a. level) between TM1300 and TM1315, while TM1323 and TM1333 are identical. Observed fold changes between *T. maritima* TSP and pure cultures are reported below the diagrams of the respective putative operons.

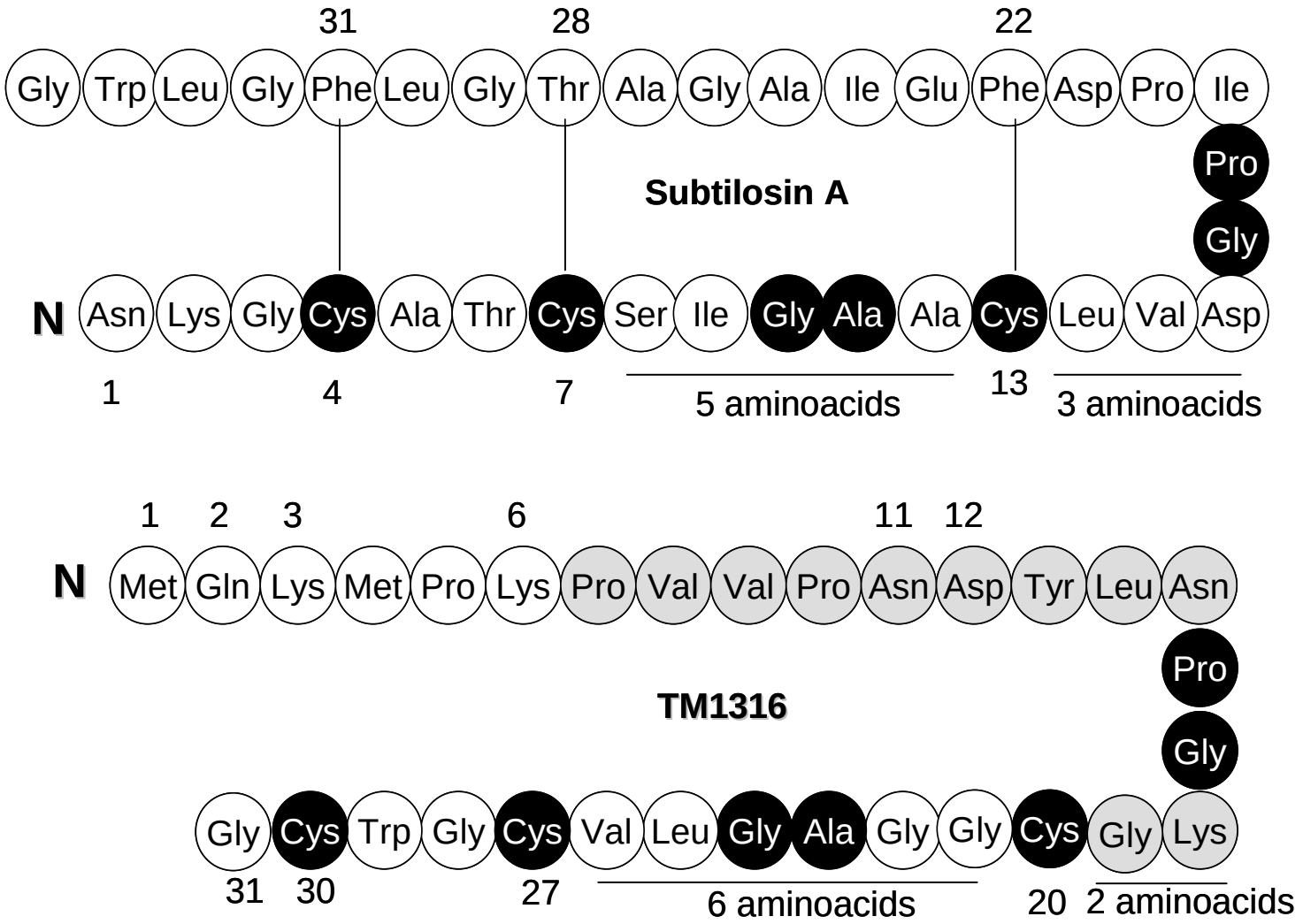


FIGURE 1.10. Comparison between the mature form of Subtilosin A and TM1316. The mature form of Subtilosin A (Top) is a bacteriocin produced by *B. subtilis* with antimicrobial activity against *Listeria* sp. The section in gray (aa 7-19) in TM1316 refers to residues previously identified in tryptic digest of the secretome of *T. maritima* by LC/MS (Johnson unpublished). Subtilosin A schematic was adapted from Thennarasu *et al.* 2005.

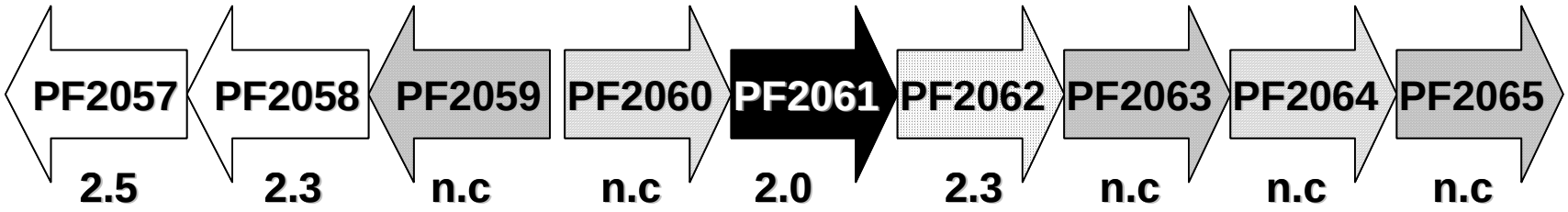


Figure 1.11. Expression of selected ORFs in *P. furiosus* adjacent to AstB/ ChuR-related genes. The number below the arrows indicates the foldChange of genes in co-culture vs. pure culture conditions in HPM at low cell density. All ORF's are coded according to their structural features and domains. Genes that are represented as a white arrow (⇨) correspond to small ORFs (<80aa). Genes represented as a dotted arrow (⇨) are members of the SAM-Radical superfamily. Shown as grey arrows (⇨) are putative aminopeptidases. (⇨) Refers to a potential transcriptional regulator with a Helix-turn-Helix motif. Represented as a black arrow (➡) PF2061 a putative permease component of an ABC transporter

SUPPLEMENTARY MATERIAL

Transcriptomes of hyperthermophiles Pyrococcus furiosus and Thermotoga maritima are influenced by co-cultivation and growth in each other's spent media

Clemente I. Montero, Derrick L. Lewis, Matthew R. Johnson, Keith R. Shockley, Elizabeth A. Nance, Shannon B. Conners, Steven R. Gray, and Robert M. Kelly

Table 1.2. Summary of differentially transcribed ORFs* in co-culture and spent media growth experiments

Condition		Up-regulated	Down-regulated	Total
HPM				
Comparison	**Harvest***			
Co-culture vs. Pfu pure culture	High cell density	**212**	**239**	**451**
Co-culture vs. Tma pure culture	High cell density	**20**	**36**	**56**
Co-culture vs. Pfu pure culture	Low cell density	**36**	**28**	**64**
Co-culture vs. Tma pure culture	Low cell density	**214**	**120**	**334**
LPM				
Comparison	**Harvest***			
Co-culture vs. Pfu pure culture	Low cell density	**25**	**43**	**68**
Co-culture vs. Tma pure culture	Low cell density	**16**	**22**	**38**
Spent Media (HPM)				
Comparison	**Harvest***			
Co-culture vs. Tma pure culture in spent Pfu	High cell density	**0**	**0**	**0**
Spent Media (LPM)				
Comparison	**Harvest***			
Pfu in spent Tma vs. Pfu pure culture	Low cell density	**53**	**55**	**108**
Co-culture vs. Pfu pure culture in spent Tma	Low cell density	**23**	**11**	**34**
Tma in spent Pfu vs. Tma pure culture	Low cell density	**34**	**5**	**39**
Co-culture vs. Tma pure culture in spent Pfu	Low cell density	**11**	**43**	**54**

*"low cell density" - harvested between 1 x 10^7-2.8 x 10^7cells/ml

*"high cell density" – harvested between 9.9 x 10^7-1.2 x 10^8cells/ml

(*) Differential transcription was defined as ORFs changing ≥ 2-fold.

TABLE 1.8. Differentially expressed ORFs for *P. furiosus* in pure and co-culture conditions in HPM at high cell density

Gene	Log (2) fold change CO vs. PFU	-log(10)p-value	Annotation (*)
PF0004	1.1	10.2	Permeases of the major facilitator
PF0014	2.0	17.9	Uncharacterized conserved protein
PF0015	1.8	21.0	Archaeal enzymes of ATP-grasp superfamily
PF0023	1.0	11.8	ATPase involved in DNArepair
PF0091	1.3	14.9	
PF0114	1.0	10.0	Predicted GTPase
PF0115	1.5	16.7	ATP-dependent 26S proteasome regulatory subunit
PF0119	1.7	17.3	Maltose-binding periplasmic proteins/domains
PF0120	1.6	4.4	
PF0121	2.3	16.1	Transcriptional regulators containing a DNA-binding domain
PF0131	1.3	16.1	
PF0137	1.1	8.5	SAM-dependent methyltransferases
PF0152	1.3	15.5	Uncharacterized conserved protein
PF0153	2.0	19.0	Phosphoribosylaminoimidazolesuccinocarboxamide (SAICAR) synthase
PF0163	1.1	13.0	Predicted membrane protein
PF0191	2.1	15.7	ABC-type dipeptide/oligopeptide/nickel transport systems, permease
PF0192	1.2	13.9	ABC-type dipeptide/oligopeptide/nickel transport systems, permease
PF0193	1.0	1.8	ABC-type dipeptide/oligopeptide/nickel transport system, ATPase
PF0234	1.5	16.6	Sec-independent protein secretion pathway component
PF0235	1.1	12.2	Nucleoside-diphosphate-sugar pyrophosphorylase involved in lipopolysaccharide synthesis
PF0239	1.0	15.0	Uncharacterized protein conserved in archaea
PF0248	1.5	16.3	Uncharacterized conserved protein
PF0249	1.3	14.3	Xanthosine triphosphate pyrophosphatase
PF0287	1.8	12.2	Subtilisin-like serine proteases
PF0321	1.0	8.5	FOG: CBS domain
PF0339	1.7	21.6	SAM-dependent methyltransferases
PF0340	1.6	17.5	Uncharacterized protein conserved in archaea
PF0341	1.5	15.5	ATPase involved in DNA repair
PF0342	1.5	19.4	
PF0352	1.5	11.9	Uncharacterized protein
PF0353	1.2	19.8	ATP-dependent DNA ligase
PF0356	1.5	14.4	Beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase
PF0381	2.1	15.2	
PF0382A	1.2	14.5	
PF0390	1.1	14.8	
PF0392	1.2	17.5	Predicted membrane-associated Zn-dependent proteases

PF0414	1.0	12.3	
PF0421	1.9	17.1	ATP-utilizing enzymes of ATP-gras P superfamily
PF0422	1.7	17.1	Phosphoribosylamine-glycine ligase
PF0430	1.9	13.6	Formate-dependent phosphoribosylglycinamide formyltransferase
PF0443	1.6	17.1	Permeases of the major facilitator
PF0444	1.6	13.7	Uncharacterized conserved protein
PF0445	1.7	19.6	Galactokinase
PF0448	1.7	19.4	Nucleoside-diphosphate-sugar pyrophosphorylase involved in lipopolysaccharides synthesis
PF0449	2.8	21.9	Predicted permeases
PF0450	1.3	15.2	Glutamine synthetase
PF0452	1.0	17.5	Uncharacterized protein conserved in archaea
PF0453	1.4	18.0	
PF0457	1.4	14.1	Zn-dependent proteases
PF0458	1.1	14.7	Nicotinamide mononucleotide adenylyltransferase
PF0471	1.5	13.5	Type II secretory pathway, pre-pilin protein
PF0472	1.4	12.3	
PF0480	1.3	13.9	Lysophospholipase
PF0485	1.2	11.7	ATPases involved in chromosome partitioning
PF0489	3.0	15.4	
PF0532	1.6	14.0	Acyl-CoA synthetase (NDP forming)
PF0533	2.5	18.4	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF0534	1.8	14.9	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF0544	1.3	12.3	Uncharacterized conserved protein
PF0545	1.1	13.8	
PF0546	1.5	15.8	
PF0547	1.1	13.0	Predicted GTPase
PF0569	1.0	9.6	Uncharacterized conserved protein
PF0578	1.8	17.2	ABC-type multidrug transport system, permease
PF0579	1.2	11.5	ABC-type Na+ efflux pump, permease
PF0580	1.2	15.6	
PF0593	1.0	13.5	Predicted hydrolase (metallo-beta-lactamase superfamily)
PF0613	1.4	15.8	Archaeal fructose 1,6-bisphosphatase
PF0641	1.0	18.7	
PF0642	1.0	17.4	Uncharacterized protein predicted
PF0653	1.1	14.1	
PF0672	1.1	11.0	
PF0673	1.1	12.1	Biotin carboxyl carrier protein
PF0674	1.0	15.5	Na+-transporting methylmalonyl-CoA/oxaloacetate decarboxylase, beta subunit
PF0677	1.7	13.7	Superfamily II helicase
PF0681	1.2	12.2	
PF0702	1.0	9.9	Xaa-Pro aminopeptidase
PF0730	1.2	14.0	
PF0746	1.1	13.4	Predicted divalent heavy-metal cation transporter
PF0747	1.1	12.9	Xaa-Pro aminopeptidase
PF0752	1.5	9.4	Peroxiredoxin
PF0753	1.2	14.0	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF0816	1.0	15.6	Small-conductance mechano-sensitive channel
PF0839	1.1	12.4	

PF0842	1.1	8.3	
PF0846	1.4	16.0	Predicted metal-dependent hydrolase
PF0852	1.4	16.9	Permeases
PF0868	1.9	18.8	Nucleoside-diphosphate-sugar pyrophosphorylase involved in lipopolysaccharide synthesis
PF0873	1.0	11.0	RecA-superfamily ATPases
PF0883	1.0	12.6	
PF0887	1.0	10.8	
PF0892	1.2	15.6	2-polyprenylphenol hydroxylase and related flavodoxin
PF0893	1.3	11.1	Coenzyme F420-reducing hydrogenase, gamma subunit
PF0894	1.0	12.4	Coenzyme F420-reducing hydrogenase, alpha-subunit
PF0895	1.7	15.4	ABC-type multidrug transport system, ATPase
PF0896	1.1	12.4	
PF0897	2.1	14.7	
PF0948	2.2	25.3	
PF0951	1.1	11.0	
PF0955	1.2	11.9	
PF0957	1.0	10.1	
PF0961	2.0	17.0	
PF0962	1.6	19.2	
PF0996	1.5	17.1	Uncharacterized protein conserved in archaea
PF1013	1.4	17.7	
PF1014	1.2	13.7	
PF1100A	1.5	10.0	
PF1105	1.2	15.1	Amidases related to nicotinamidase
PF1136	1.3	14.8	ABC-type sugar transport system, periplasmic
PF1152	1.2	7.5	Multisubunit Na+/H+ antiporter, MnhFsubunit
PF1196	1.5	13.1	Uncharacterized conserved protein
PF1197	1.4	20.5	Uncharacterized NAD(FAD)-dependent dehydrogenases
PF1206	1.2	13.6	
PF1216	1.1	15.0	Putative translation factor (SUA5)
PF1223	1.0	9.3	Predicted nucleic acid-binding protein.
PF1245	1.0	14.4	Uncharacterized NAD(FAD)-dependent dehydrogenases
PF1246	1.1	13.7	Glycine/D-amino acid oxidases (deaminating)
PF1250	1.8	16.3	
PF1251	1.9	16.5	S-layer domain
PF1252	1.3	15.9	ABC-type transport system
PF1270	1.2	13.8	
PF1283	1.2	10.4	Rubrerythrin
PF1303	1.2	15.7	
PF1304	1.6	14.6	
PF1305	1.3	14.3	
PF1306	2.8	19.6	
PF1307	1.1	13.3	
PF1310	1.6	14.0	
PF1314	1.0	11.8	Uncharacterized conserved protein
PF1315	1.2	7.2	Uncharacterized conserved protein
PF1339	1.9	16.4	
PF1340	1.7	15.2	
PF1395	1.1	14.6	Phosphate transport regulator
PF1396	1.3	10.1	Ketopantoate reductase
PF1408	1.0	11.1	ABC-type dipeptide transport system, periplasmic
PF1423	1.4	17.3	Multisubunit Na+/H+ antiporter, MnhE subunit
PF1424	1.6	17.1	Multisubunit Na+/H+ antiporter, MnhF subunit

PF1425	1.6	16.6	Multisubunit Na+/H+ antiporter, MnhG subunit
PF1426	1.2	11.4	
PF1427	1.1	12.4	Multisubunit Na+/H+ antiporter, MnhB subunit
PF1428	1.5	13.7	Multisubunit Na+/H+ antiporter, MnhB subunit
PF1429	1.4	12.5	Multisubunit Na+/H+ antiporter, MnhC subunit
PF1431	1.1	12.5	
PF1433	1.6	14.3	NADH:ubiquinone oxidoreductase 27 kDsubunit
PF1434	1.8	19.4	Ni,Fe-hydrogenase III large subunit
PF1435	2.1	19.2	Formate hydrogen lyase subunit 4
PF1436	1.0	0.9	Formate hydrogen lyase subunit 6/NADH:ubiquinoneoxidoreductase
PF1438	1.3	21.6	Archaeal serine proteases
PF1441	1.9	18.1	Formate hydrogenlyase subunit 6/NADH:ubiquinoneoxidoreductase
PF1442	1.6	16.5	NADH:ubiquinone oxidoreductase 49 kDsubunit
PF1443	1.7	16.7	NADH:ubiquinone oxidoreductase 27 kDsubunit
PF1444	1.7	14.8	NADH:ubiquinone oxidoreductase 20 kDsubunit
PF1445	1.7	17.6	Formate hydrogen lyase subunit 4
PF1446	1.8	18.0	Formate hydrogen lyase subunit 3/MultisubunitNa+/H+
PF1448	1.5	17.3	Multisubunit Na+/H+ antiporter, MnhC subunit
PF1451	1.1	12.5	Multisubunit Na+/H+ antiporter, MnhG subunit
PF1456	1.2	15.7	Histone acetyltransferase HPA2 andrelated
PF1479	1.1	8.2	Fe-S-cluster-containing hydrogenase components 2
PF1482	2.0	19.6	
PF1494	1.3	17.8	
PF1497	1.6	16.4	Aspartate/tyrosine/aromatic aminotransferase
PF1508	1.1	13.2	
PF1509	1.7	16.0	
PF1510	1.6	15.3	
PF1511	1.0	12.2	
PF1533	1.8	11.3	Membrane-bound serine protease (Clp P class)
PF1534	1.2	15.4	Membrane protease subunits, stomatin/prohibitin homologs
PF1543	1.5	14.2	Transcriptional regulators
PF1544	1.1	15.8	
PF1548	1.2	15.2	
PF1549	1.3	13.8	RNA 3'-terminal phosphate cyclase
PF1562	2.1	20.4	DNA-directed RNA polymerase, beta subunit/160
PF1563	2.5	20.6	DNA-directed RNA polymerase, beta subunit/160
PF1564	2.4	23.0	DNA-directed RNA polymerase, beta subunit/140
PF1572	1.7	16.2	Predicted regulator of aminoacid synthesis
PF1573	2.6	19.2	Predicted Zn-dependent proteases
PF1574	1.0	13.0	Predicted Zn-dependent proteases
PF1614	1.0	14.8	Predicted ATPase involved inreplication
PF1628	1.5	14.5	Predicted Fe-S oxidoreductase
PF1632	1.0	8.6	
PF1635	1.0	9.5	ATP-dependent DNA ligase
PF1652	1.3	4.7	
PF1653	1.0	8.6	
PF1668	1.6	16.1	
PF1669	1.1	12.0	
PF1689	1.2	16.8	Transketolase, C-terminal subunit
PF1696	1.9	15.8	ABC-type uncharacterized transport systems, ATPase
PF1723	1.6	18.3	Fe-S oxidoreductase

PF1727	1.6	14.8	
PF1757	1.0	15.1	
PF1775	1.1	10.4	Predicted membrane protein
PF1777	1.1	12.6	Predicted hydrolase (HAD superfamily)
PF1778	1.5	13.8	Glycine/serine hydroxymethyltransferase
PF1796	1.0	6.7	Fe-S-cluster-containing hydrogenase components 2
PF1797	1.0	11.9	Uncharacterized NAD(FAD)-dependent dehydrogenases
PF1828	1.1	13.1	
PF1832	1.8	13.7	Uncharacterized conserved protein
PF1836	1.0	8.7	
PF1838	1.1	13.1	Acyl-CoA synthetase (NDP forming)
PF1851	1.2	12.0	Predicted transcriptional regulator
PF1875	1.1	15.3	
PF1876	1.6	15.3	
PF1877	1.2	14.4	Thiamine monophosphate kinase
PF1894	1.8	15.7	Signal recognition particle 19kDa
PF1897	1.1	11.8	Transcriptional regulators
PF1898	1.5	20.4	Predicted Fe-S-cluster oxidoreductase
PF1904	1.2	13.4	Nicotinic acid phosphoribosyltransferase
PF1905	1.5	14.6	Molecular chaperone
PF1906	1.4	12.0	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase
PF1907	2.4	20.7	
PF1908	1.6	13.9	
PF1916	1.0	12.0	Glycosyltransferases
PF1917	1.2	11.9	Predicted dehydrogenases and related proteins
PF1940	1.2	11.7	Protein involved in ribosomal biogenesis
PF1947	1.0	8.9	Putative periplasmic protein kinase ArgK
PF1949	1.0	14.3	
PF1950	1.2	9.0	Predicted phosphoribosyltransferases
PF1951	1.1	13.0	Aspartyl/asparaginyl-tRNA synthetases
PF1960	1.1	8.6	Aldo/keto reductases, related to diketogulonate
PF1961	1.1	14.6	Aldehyde:ferredoxin oxidoreductase
PF1967	2.2	13.5	ABC-type sugar transport system, periplasmic
PF1968	1.4	13.7	ABC-type sugar transport systems, permease
PF1970	1.3	14.9	ABC-type sugar transport systems, ATPase
PF1971	1.0	13.5	Oxygen-sensitive ribonucleoside-triphosphate reductase
PF1999	3.4	19.5	Glycine cleavage system protein P
PF2000	3.7	21.3	Glycine cleavage system protein P
PF2001	1.1	12.3	Hydrolases of the alpha/beta-superfamily
PF2002	1.0	7.7	Permeases of the major facilitator
PF2061	1.4	11.6	ABC-type multi-drug transport system, permease
PF2062	1.0	7.7	Transcriptional regulators
PF0008	-1.2	14.1	Diadenosine tetraphosphate (Ap4A) hydrolase
PF0009	-1.0	11.5	Dinucleotide-utilizing enzymes involved in molybdopterin thiamine biosynthesis
PF0010	-1.4	12.1	1-aminocyclopropane-1-carboxylate deaminase
PF0011	-1.0	13.4	Predicted membrane protein
PF0032	-2.3	18.8	Permeases of the major facilitator
PF0043	-1.6	17.9	Phosphoenolpyruvate synthase/pyruvate phosphate di-kinase
PF0070	-1.1	13.9	Uncharacterized archaeal coiled-coil protein
PF0071	-1.2	12.1	Queuine tRNA-ribosyltransferases, contain PUA domain
PF0075	-1.2	15.3	Alcohol dehydrogenase, class IV

PF0086	-1.0	13.9	Predicted metal-dependent hydrolases
PF0094	-1.5	14.5	Thiol-disulfide isomerase and thioredoxins
PF0132	-1.5	18.7	
PF0133	-1.5	18.5	Uncharacterized conserved protein
PF0155	-2.0	21.3	Aspartyl/asparaginyl-tRNA synthetases
PF0172	-1.1	13.7	o-sialoglycoprotein endopeptidase
PF0176	-1.3	12.5	
PF0178	-1.3	14.6	F0F1-type ATP synthase, subunitc/Archaeal/vacuolar-type
PF0183	-1.0	14.2	Archaeal/vacuolar-type H+-ATPase subunit B
PF0185	-1.1	10.3	Predicted metal-dependent hydrolases
PF0196	-1.0	11.7	Thermophilic glucose-6-phosphate isomerase andrelated
PF0205	-1.5	15.9	Glutamate synthase domain 2
PF0206	-2.6	23.6	Glutamate synthase domain 3
PF0207	-2.6	22.3	Argininosuccinate synthase
PF0208	-1.5	15.4	Argininosuccinate lyase
PF0215	-1.8	17.0	Enolase
PF0217	-2.5	19.1	Ribosomal protein L44E
PF0253	-1.7	16.8	Ribosomal protein S24E
PF0254	-1.3	16.6	Uncharacterized protein conserved in archaea
PF0257	-1.6	15.1	Inorganic pyrophosphatase
PF0264	-1.4	17.1	Histidyl-tRNA synthetase
PF0276	-2.5	18.7	Uncharacterized FAD-dependent dehydrogenases
PF0284	-1.1	16.8	
PF0285	-1.6	15.6	IMP dehydrogenase/GMP reductase
PF0308	-1.4	5.0	Adenylosuccinate synthase
PF0312	-1.3	17.5	
PF0343	-1.0	12.0	S-adenosylhomocysteine hydrolase
PF0346	-1.2	13.5	Aldehyde:ferredoxin oxidoreductase
PF0366	-1.1	11.6	Dephospho-CoA kinase
PF0369	-1.1	12.1	Cellulase M and related proteins
PF0375	-1.0	16.6	Predicted prefoldin, molecular chaperone
PF0376	-1.8	14.2	
PF0377	-1.4	11.8	Translation initiation factor 6 (eIF-6)
PF0378	-1.7	19.2	Ribosomal protein L31E
PF0379	-1.4	13.3	
PF0406	-1.1	8.3	FOG: EAL domain
PF0407	-1.2	12.4	
PF0432	-1.5	15.2	Predicted sugar phosphatases
PF0463	-1.2	19.0	Predicted hydrolase (HAD superfamily)
PF0488	-2.5	20.0	Ribosomal protein S6E (S10)
PF0499	-1.0	14.2	Arginase/agmatinase/formimionoglutamate hydrolase, arginase family
PF0500	-1.1	16.9	Predicted methyltransferase
PF0501	-1.1	14.8	Predicted nucleotide kinase
PF0510	-1.1	13.6	Predicted pyrophosphatase
PF0531	-1.0	11.8	ABC-type Co2+ transport system, permease
PF0556	-1.2	10.4	
PF0557	-1.8	22.0	Dehydrogenases
PF0572	-1.1	11.9	Superfamily I DNA and RNA helicase
PF0615	-1.2	13.6	Zn finger protein HypA/HybF (possibly hydrogenase)
PF0669	-1.8	16.5	Histone acetyltransferase HPA2 andrelated
PF0670	-1.2	14.4	Predicted ATPase, RNase L inhibitor
PF0678	-1.3	13.7	Uncharacterized conserved protein
PF0716	-1.5	19.2	3-hydroxyisobutyrate dehydrogenase and relatedbeta-

			hydroxyacid
PF0722	-1.0	10.5	Peroxiredoxin
PF0765	-1.0	12.1	UDP-N-acetyl-D-mannosaminuronate dehydrogenase
PF0768	-1.2	13.0	Acetyltransferase (isoleucine patch superfamily)
PF0806	-1.0	16.0	FOG: EAL domain
PF0808	-1.3	15.8	
PF0810	-1.0	8.4	NhaP-type Na+/H+ and K+/H+antiporters
PF0819	-1.9	18.0	Ribosomal protein L14E/L6E/L27E
PF0820	-1.3	11.4	Cytidylate kinase
PF0821	-1.3	20.2	Ribosomal protein L34E
PF0822	-1.1	12.3	
PF0824	-1.8	15.0	Na+-driven multidrug efflux pump
PF0825	-1.7	13.4	Prolyl endopeptidase
PF0829	-1.6	15.1	
PF0835	-1.1	12.3	ABC-type multidrug transport system ATPase
PF0849	-1.3	15.5	Fe-S oxidoreductase, related to NifB/MoaA
PF0859	-1.6	11.6	Mg-dependent DNase
PF0876	-2.0	20.3	Ribosomal protein L15E
PF0929	-1.9	15.3	
PF0930	-1.6	13.9	Uncharacterized Rossmann fold enzyme
PF0935	-1.6	16.2	Thiamine pyrophosphate-requiring enzymes acetolactate synthase
PF0936	-3.2	18.8	Ketol-acid reductoisomerase
PF0937	-2.5	20.0	Isopropylmalate/homocitrate/citramalate synthases
PF0938	-1.5	14.9	3-isopropylmalate dehydratase large subunit
PF0939	-1.2	10.6	3-isopropylmalate dehydratase small subunit
PF0940	-1.9	17.1	Isocitrate/isopropylmalate dehydrogenase
PF0941	-2.1	14.7	Isopropylmalate/homocitrate/citramalate synthases
PF0942	-2.3	17.2	Dihydroxyaciddehydratase/phosphogluconate dehydratase
PF0974	-1.2	12.7	Predicted nucleic-acid-binding protein
PF0983	-1.3	10.5	DNA polymerase sliding clamp subunit
PF0984	-1.9	16.1	
PF0989	-1.1	13.7	Phenylalanyl-tRNA synthetase alpha subunit
PF0990	-1.4	13.5	Phenylalanyl-tRNA synthetase beta subunit
PF1021	-1.7	11.0	Phosphate transport regulator
PF1032	-1.4	16.7	NMD protein affecting ribosome estability
PF1033	-1.0	16.3	Peroxiredoxin
PF1035	-1.9	15.8	Ribosomal protein L21E
PF1036	-1.2	11.5	DNA-directed RNA polymerase, subunit F
PF1043	-1.5	16.9	RecA-superfamily ATPases
PF1051	-1.3	11.0	Lhr-like helicases
PF1053A	-2.6	17.2	
PF1053B	-2.9	17.9	
PF1054	-2.2	15.7	Homoserine kinase
PF1055	-2.2	19.0	Threonine synthase
PF1056	-1.4	14.9	Aspartate-semialdehyde dehydrogenase
PF1062	-2.8	19.8	Predicted RNA-binding protein
PF1063	-1.2	13.7	Diphthamide synthase subunit DPH2
PF1069A	-1.9	14.5	
PF1069B	-1.8	17.4	
PF1096	-2.3	20.6	Isoleucyl-tRNA synthetase
PF1102	-2.1	20.3	Geranylgeranyl pyrophosphate synthase
PF1104	-1.2	17.9	Homoserine dehydrogenase
PF1109	-1.4	13.8	

PF1110	-1.2	12.7	
PF1133	-1.0	8.3	
PF1139	-1.0	13.1	Predicted pseudouridylate synthase
PF1145	-1.7	16.1	ATPases involved in chromosome partitioning
PF1172	-1.2	11.5	SAM-dependent methyltransferases
PF1203	-1.0	9.9	Aldehyde:ferredoxin oxidoreductase
PF1211	-1.5	13.5	ABC-type dipeptide/oligopeptide/nickel transport systems, permease
PF1237	-1.2	15.1	Uncharacterized conserved protein
PF1239	-1.0	12.7	
PF1242	-1.0	11.9	Anaerobic dehydrogenases.
PF1243	-1.4	15.2	
PF1258	-1.4	12.0	Ribose 5-phosphate isomerase
PF1264	-1.6	14.2	Translation elongation factor P (EF-P)
PF1265	-1.1	11.9	tRNA and rRNA cytosine-C5-methylases
PF1278	-1.4	19.2	6-pyruvoyl-tetrahydropterin synthase
PF1279	-2.9	20.8	Ribosomal protein L16/L10E
PF1284	-1.8	20.8	
PF1285	-1.0	10.6	ABC-type transport system
PF1286	-1.9	13.3	ABC-type transport system
PF1289	-2.1	13.7	Dinucleotide-utilizing enzymes
PF1293	-2.1	20.4	Prolyl-tRNA synthetase
PF1295	-1.4	19.0	TATA-box binding protein (TBP)
PF1296	-1.9	16.2	Dihydropteroate synthase and related enzymes
PF1330	-2.4	17.1	2-polyprenylphenol hydroxylase and related flavodoxin
PF1331	-1.3	19.3	Coenzyme F420-reducing hydrogenase, gamma subunit
PF1332	-1.8	16.9	Coenzyme F420-reducing hydrogenase, alpha subunit
PF1345	-1.7	19.7	Metal-dependent hydrolases of the beta-lactamase
PF1347	-1.5	19.9	
PF1351	-1.0	14.5	Threonyl-tRNA synthetase
PF1367	-1.1	8.5	Ribosomal protein HS6-type (S12/L30/L7a)
PF1368	-1.2	15.3	Ribosomal protein S28E/S33
PF1369	-1.4	14.7	Ribosomal protein L24E
PF1375	-1.8	20.7	GTPases - translation elongation factors
PF1376	-2.1	18.3	Ribosomal protein S10
PF1418	-1.0	11.0	Uncharacterized protein conserved in archaea
PF1462	-1.4	14.7	Archaeal Glu-tRNA Gln amidotransferase subunit E
PF1480	-1.4	18.3	Aldehyde:ferredoxin oxidoreductase
PF1485	-1.1	8.8	Uncharacterized protein conserved in archaea
PF1499	-1.2	12.7	Ribosomal protein S19E (S16A)
PF1538	-1.4	10.3	Cytosine deaminase and related metal-dependent
PF1540	-1.0	12.6	Acyl-CoA synthetase (NDP forming)
PF1542	-1.0	13.9	Small nuclear ribonucleoprotein (snRNP) homolog
PF1558	-1.8	11.0	Ribosomal protein S7
PF1559	-1.6	19.9	Ribosomal protein S12
PF1560	-1.5	7.4	Transcription elongation factor
PF1561	-2.1	16.5	Ribosomal protein L30E
PF1565	-2.3	20.3	DNA-directed RNA polymerase, subunit H
PF1567	-1.1	12.5	RNase PH-related exoribonuclease
PF1588	-1.1	13.4	Uncharacterized conserved protein
PF1591	-1.4	15.2	D-aminopeptidase
PF1592A	-1.9	16.6	
PF1592B	-1.3	13.3	
PF1602	-2.8	21.2	Glutamate dehydrogenase/leucine dehydrogenase

PF1623	-1.2	14.1	Uncharacterized conserved protein
PF1627	-1.9	7.2	Glycyl-tRNA synthetase (class II)
PF1640	-1.2	14.1	Ribosomal protein S2
PF1644	-1.9	18.5	Ribosomal protein S9
PF1645	-1.6	18.4	Ribosomal protein L13
PF1646	-1.6	14.5	Ribosomal protein L18E
PF1648	-1.6	13.5	Ribosomal protein S11
PF1649	-1.3	13.1	Ribosomal protein S4 andrelated
PF1650	-1.4	15.8	Ribosomal protein S13
PF1657	-1.2	14.4	Histidyl-tRNA synthetase
PF1658	-1.3	15.0	ATP phosphoribosyltransferase
PF1659	-1.5	15.4	Histidinol dehydrogenase
PF1660	-1.0	11.0	Imidazoleglycerol-phosphate dehydratase
PF1663B	-1.0	17.6	
PF1681	-1.0	11.7	
PF1687	-1.3	5.9	
PF1688	-1.2	14.9	Transketolase
PF1690	-1.0	10.8	3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase
PF1693	-1.1	13.4	Shikimate 5-dehydrogenase
PF1704	-1.0	8.7	
PF1713	-3.4	22.4	Carbamoylphosphate synthase small subunit
PF1714	-1.6	13.7	Carbamoylphosphate synthase large subunit
PF1715	-2.1	19.6	Pyrroline-5-carboxylate reductase
PF1717	-1.4	13.0	GTPases - translation elongation factors
PF1724	-1.3	18.9	Small primase-like proteins
PF1739	-1.9	19.1	ABC-type sugar transport system, periplasmic
PF1742	-1.8	13.9	Glycosyltransferase
PF1744	-1.0	12.5	ABC-type sugar transport systems, ATPase
PF1745	-1.2	12.1	L-fucose isomerase
PF1746	-1.7	14.0	Glycogen debranching enzyme
PF1747	-1.7	16.9	Beta-fructosidases (levanase/invertase)
PF1758	-1.4	14.9	Predicted ATPase of thePP-loop
PF1783	-2.0	15.7	Molybdopterin biosynthesis enzyme
PF1784	-1.9	15.7	
PF1786	-1.1	15.4	
PF1800	-1.0	14.1	Archaeal adenylate kinase
PF1802	-1.5	16.8	Ribosomal protein L15
PF1803	-1.5	16.3	Ribosomal protein L30/L7E
PF1804	-1.2	13.2	Ribosomal protein S5
PF1805	-1.4	17.0	Ribosomal protein L18
PF1806	-1.5	19.7	Ribosomal protein L19E
PF1807	-1.6	13.1	Ribosomal protein L32E
PF1808	-1.2	15.5	Ribosomal protein L6P/L9E
PF1809	-1.4	20.1	Ribosomal protein S8
PF1810	-1.2	10.1	Ribosomal protein S14
PF1811	-1.8	15.3	Ribosomal protein L5
PF1812	-1.4	7.4	Ribosomal protein S4E
PF1813	-1.3	15.9	Ribosomal protein L24
PF1814	-1.4	3.9	Ribosomal protein L14
PF1815	-1.4	9.9	Ribosomal protein S17
PF1817	-1.5	15.1	Translation initiation factor 1(eIF-1/SUI1)
PF1818	-1.5	19.6	Ribosomal protein L29
PF1819	-1.6	16.0	

TABLE 1.9. Differentially expressed ORFs for *T. maritima* in pure and co-culture conditions in HPM at high cell density			
Gene	**Log (2) fold change CO vs. TMA**	**-log(10)p-value**	**Annotation (*)**
TM0035	1.0	13.7	Hypothetical protein
TM0036	1.0	10.5	Conserved hypothetical
TM0165	1.0	11.9	Holliday junction DNA recombination protein
TM0384	1.8	17.7	Anaerobic ribonucleosidase
TM0385	1.4	17.4	Conserved hypothetical
TM0560	1.5	19.2	Conserved hypothetical
TM0606	1.3	17.2	Hypothetical protein
TM0659	1.2	12.7	Rubredoxin
TM0696	1.1	13.1	Ray-related protein
TM0715	1.1	11.2	tRNA nucleotidyl transferase
TM0848	1.0	8.7	Conserved hypothetical
TM0870	1.3	7.7	Penicillin-binding protein
TM1027	1.1	8.9	Hypothetical protein
TM1167	1.4	18.9	Hypothetical protein
TM1246	1.0	17.8	Phosphoribosylformylglycinamide synthase
TM1266	1.2	18.5	Hypothetical protein
TM1267	1.0	10.7	ThiH protein, putative
TM1685	1.3	18.5	Conserved hypothetical
TM1742	1.0	7.9	NagD protein, putative
TM1874	1.4	11.3	Cold shock protein
TM_tRNA	-1.0	1.2	
TM0044	-1.0	11.0	Hypothetical protein
TM0050	-1.7	18.7	Iron (II) transport protein
TM0051	-1.2	13.9	Iron (II) transport protein
TM0121	-2.0	14.1	Conserved hypothetical
TM0315	-1.1	12.8	Hypothetical protein
TM0338	-1.4	11.7	Hypothetical protein
TM0359	-1.5	15.3	Conserved hypothetical
TM0373	-1.9	16.9	DnaK protein
TM0417	-1.3	11.0	Conserved hypothetical
TM0505	-2.0	16.5	GroES protein
TM0506	-1.5	15.5	GroEL protein
TM0616	-1.4	16.9	Conserved hypothetical
TM0654	-1.2	10.6	Spermidine synthase
TM0823	-1.2	9.9	Transcriptional regulator
TM0824	-1.4	11.4	AstB/chuR-related protein
TM0826	-1.1	12.3	Hypothetical protein
TM0849	-1.0	11.0	DnaJ protein
TM0850	-2.1	14.7	GrpE protein, putative
TM0916	-1.5	10.4	Conserved hypothetical
TM0918	-1.2	15.0	Methyl-accepting chemotaxis protein
TM0974	-1.1	9.4	Hypothetical protein
TM0975	-1.3	13.2	Hypothetical protein
TM0979	-1.5	23.6	Conserved hypothetical
TM0980	-2.0	16.1	Uncharacterized protein

TM0981	-2.2	19.3	Uncharacterized ACR
TM0982	-2.4	17.2	Conserved hypothetical
TM0983	-1.7	1.9	Conserved hypothetical
TM0989	-1.1	13.7	Conserved hypothetical
TM1015	-1.9	18.2	Glutamate dehydrogenase
TM1144	-1.4	11.4	Hypothetical protein
TM1145	-1.6	18.5	Conserved hypothetical
TM1232	-1.0	0.8	Sugar ABC transporter
TM1235	-1.2	10.9	Conserved hypothetical
TM1375	-1.6	14.5	Spermidine/putrescine transporter protein
TM1418	-1.1	14.6	

(*) Original annotation (57)

TABLE 1.10. Differentially expressed ORFs for *P. furiosus* in co-culture and pure culture on HPM at low cell density			
Gene	**Log (2) fold change CO vs. PFU**	**-log(10)p-value**	**Annotation (*)**
PF0182	1.1	5.4	Archaeal/vacuolar-type H+-ATPase subunit A
PF0200	1.1	16.6	Uncharacterized conserved protein
PF0201	1.6	16.7	Aconitase A
PF0202	1.4	12.9	Isocitrate dehydrogenases
PF0203	1.4	17.8	Citrate synthase
PF0204	1.1	14.1	Glutamate synthase domain 1
PF0205	1.0	12.2	Glutamate synthase domain 2
PF0243	1.0	7.3	Predicted ATPase (AAA+ superfamily)
PF0244	1.3	9.3	
PF0291	1.5	4.3	Prephenate dehydratase
PF0336	1.4	7.2	Putative archaeal flagellar protein C
PF0430	1.1	11.1	Formate-dependent phosphoribosylglycinamide formyltransferase (GAR-transferase)
PF0433	1.1	10.3	
PF0449	1.0	9.0	Predicted permeases
PF0566	1.0	7.7	Predicted ATPase (AAA+ superfamily)
PF0575	1.1	11.6	Predicted nucleic acid-binding protein
PF0651	1.4	12.3	
PF0661	1.3	9.7	Predicted transcriptional regulators
PF0664	1.1	8.7	
PF0677	1.7	10.7	Superfamily II helicase
PF0702	1.1	9.5	Xaa-Pro aminopeptidase
PF0729	1.0	11.8	Ferredoxin
PF0730	1.5	10.0	
PF0763	1.3	11.1	
PF0781	1.2	8.3	Predicted nucleic acid-binding protein
PF0830	1.1	7.6	Membrane protein
PF0842	1.2	8.0	
PF0957	1.0	10.5	
PF0961	1.2	13.6	
PF1216	1.6	15.0	Putative translation factor (SUA5)
PF1319	1.1	8.6	Uncharacterized conserved protein
PF1465	1.1	13.2	Permeases of the major facilitator
PF1524	1.3	6.2	
PF1527	1.0	8.8	
PF1535	1.2	11.5	Glucan phosphorylase
PF1536	1.0	11.6	Predicted phosphatase
PF1632	1.7	7.8	
PF1670	1.0	9.4	Subtilisin-like serine proteases
PF1677	1.0	13.4	Uncharacterized conserved protein
PF1678	1.8	16.9	Isopropylmalate/homocitrate/citramalate

			synthases
PF1679	1.8	18.6	3-isopropylmalate dehydratase large subunit
PF1680	1.9	15.7	3-isopropylmalate dehydratase small subunit
PF1681	1.0	14.2	
PF1738	1.0	9.7	
PF1780	1.0	1.9	ABC-type Mn2+/Zn2+ transport systems, permease
PF1791	1.0	7.6	RNA-binding protein involved in rRNA modification
PF2057	1.3	10.0	
PF2058	1.2	10.6	
PF2061	1.0	6.0	ABC-type multidrug transport system, permease
PF2062	1.2	6.3	Transcriptional regulators
PF0193	-1.0	14.0	ABC-type dipeptide/oligopeptide/nickel transport system, ATPase
PF0261	-1.3	9.8	S-layer domain
PF0819	-1.0	8.6	Ribosomal protein L14E/L6E/L27E
PF0876	-1.6	18.8	Ribosomal protein L15E
PF0891	-1.1	14.1	Ferredoxin
PF0893	-1.2	16.3	Coenzyme F420-reducing hydrogenase, gamma subunit
PF0894	-1.2	16.1	Coenzyme F420-reducing hydrogenase, alpha subunit
PF0936	-1.7	11.1	Ketol-acid reductoisomerase
PF0938	-1.0	15.5	3-isopropylmalate dehydratase large subunit
PF0940	-1.6	14.7	Isocitrate/isopropylmalate dehydrogenase
PF0941	-1.7	15.6	Isopropylmalate/homocitrate/citramalate synthases
PF0942	-1.5	18.7	Dihydroxyaciddehydratase/phosphogluconate dehydratase
PF0972	-1.1	11.1	3-hydroxy-3-methylglutaryl CoA synthase
PF0974	-1.2	13.9	Predicted nucleic-acid-binding protein
PF1033	-1.2	15.6	Peroxiredoxin
PF1053A	-1.1	15.5	
PF1053B	-1.3	11.3	
PF1062	-1.0	16.2	Predicted RNA-binding protein, containsTRAM
PF1279	-1.0	12.3	Ribosomal protein L16/L10E
PF1287	-1.6	16.8	ABC-type transport system
PF1291	-1.1	16.6	Diadenosine tetraphosphatase and related serine/threonine
PF1347	-1.6	17.9	
PF1368	-1.1	12.7	Ribosomal protein S28E/S33
PF1421	-1.4	19.1	4-aminobutyrate aminotransferase and related aminotransferases
PF1499	-1.0	9.4	Ribosomal protein S19E (S16A)
PF1592A	-1.0	16.0	
PF1768	-1.3	12.6	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF1769	-1.3	13.6	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin

PF1770	-1.0	13.5	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF1772	-1.4	13.6	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF1773	-1.2	16.0	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF1786	-1.2	14.7	
PF1823	-1.1	12.9	Ribosomal protein L23
PF1975	-1.3	12.5	Uncharacterized protein conserved in archaea

(*) Annotation for *P. furiosus* genes refers to the functional role assigned to their respective COGs (76).

TABLE 1.11. Differentially expressed ORFs for *T. maritima* grown in co-culture and pure culture on HPM media at low cell density

Gene	Log (2) fold change CO vs. TMA	-log(10)p-value	Annotation (*)
TM_rnpB	-3.6	18.1	
TM_tRNA	-1.5	9.1	
TM_tRNA	-1.4	6.6	
TM_tRNA	-1.0	8.5	
TM0034	-1.0	9.7	Iron-sulfur cluster-binding protein
TM0043	-1.1	12.1	ABC transporter
TM0044	-1.6	13.1	Hypothetical protein
TM0056	-1.0	12.1	Oligopeptide ABC transporter
TM0057	-1.0	9.5	Oligopeptide ABC transporter
TM0060	-1.0	7.5	Oligopeptide ABC transporter
TM0070	-1.2	9.6	Endo-1,4-beta-xylanase
TM0080	-1.2	14.1	Iron (III) ABC transport
TM0118	-1.2	14.7	Ribonucleotide reductase
TM0122	-1.1	11.5	Ferric uptake regulation protein
TM0155	-1.2	14.5	Chorismate mutase/prephrenate synthesis
TM0156	-1.0	7.0	Alkaline phosphatase
TM0162	-1.1	9.0	Hypothetical protein
TM0179	-1.8	15.3	Hypothetical protein
TM0182	-1.2	11.3	Conserved hypothetical
TM0188	-1.2	14.2	Conserved hypothetical
TM0192	-2.1	19.9	SpoVS-related protein
TM0196	-1.7	13.0	Conserved hypothetical
TM0228	-1.4	9.0	NADP-reducing hydrogenase
TM0233	-1.4	16.0	Cell division protein,
TM0234	-1.1	11.6	UDP-N-acetylmuramoyl- ligase
TM0246	-1.1	11.6	Hypothetical protein
TM0266	-1.9	17.7	DNA-binding protein, HU protein
TM0277	-1.5	11.3	
TM0278	-1.3	11.8	Sugar ABC transporter
TM0279	-1.7	17.9	Sugar ABC transporter
TM0280	-1.7	14.7	Hypothetical protein
TM0282	-1.3	10.7	Aldose 1-epimerase
TM0283	-1.5	14.1	Sugar isomerase
TM0287	-1.1	10.2	ABC transporter, ATP binding protein
TM0296	-1.3	14.0	Fructokinase
TM0302	-1.7	12.7	Oligopeptide ABC transporter
TM0307	-1.2	12.8	L-fucose isomerase, putative
TM0335	-1.0	11.4	Dihydroorotase
TM0336	-1.2	10.7	Conserved hypothetical
TM0338	-1.2	15.3	Cypothetical protein
TM0345	-1.0	13.2	3-phosphoshikimate-1-Carboxyvinyltransferase
TM0356	-1.5	11.4	Threonine dehydratase
TM0358	-1.1	10.3	Conserved hypothetical
TM0363	-1.2	12.5	Fibronectin-binding protein

TM0368	-1.5	13.8	Permeases of the major
TM0372	-1.1	10.3	Cation efflux system protein
TM0378	-1.1	10.5	
TM0386	-1.0	15.0	
TM0412	-1.3	15.2	Alcohol dehydrogenase,
TM0413	-1.3	11.6	Creatinine amidohydrolase
TM0414	-1.5	16.2	Dehydrogenase
TM0416	-1.1	7.4	D-tagatose 3-epimerase
TM0419	-1.3	15.4	Sugar ABC transporter
TM0425	-1.1	12.0	Oxidoreductase, putative
TM0432	-1.3	9.7	Sugar ABC transporter
TM0450	-1.4	15.5	Hypothetical protein
TM0451	-1.3	11.1	Ribosomal protein L33
TM0481	-1.4	17.6	Conserved hypothetical
TM0487	-1.2	15.1	Conserved hypothetical
TM0504	-1.3	10.3	Hypothetical protein
TM0515	-1.5	16.9	Conserved hypothetical
TM0537	-1.0	7.5	Hypothetical protein
TM0563	-1.0	16.6	Conserved hypothetical
TM0572	-1.1	14.4	Lipopolysaccharide biosynthetic protein
TM0578	-1.5	12.2	Pyrroline-5-carboxylate
TM0592	-1.1	15.1	Amino acid ABC transporter
TM0593	-1.1	11.9	Amino acid ABC transporter
TM0594	-1.3	15.0	Conserved hypothetical
TM0595	-1.2	14.5	Sugar ABC transporter
TM0606	-1.7	21.3	Hypothetical protein
TM0638	-1.2	3.2	Polysaccharide export protein
TM0705	-1.0	12.9	ABC transporter
TM0728	-1.2	13.4	Conserved hypothetical
TM0729	-1.5	15.3	(p)ppGpp synthetase
TM0807	-1.7	17.5	Alkyl hydroperoxide reductase
TM0810	-1.1	15.3	Sugar ABC transporter
TM0811	-1.4	13.3	Sugar ABC transporter
TM0815	-1.3	15.3	Conserved hypothetical
TM0817	-1.2	12.9	Hypothetical protein
TM0852	-1.2	12.8	Conserved hypothetical
TM0927	-1.8	12.9	Ferredoxin
TM0935	-1.1	15.6	Conserved hypothetical
TM0936	-1.0	15.9	Conserved hypothetical
TM0941	-1.0	8.8	Hypothetical protein
TM0946	-1.3	13.0	Hypothetical protein
TM0947	-1.1	13.5	Hypothetical protein
TM0959	-1.1	10.6	Ribose ABC transporter
TM1006	-1.0	9.2	Oxidoreductase
TM1010	-1.5	10.7	Hypothetical protein
TM1011	-1.3	12.6	Conserved hypothetical
TM1030	-1.1	11.9	Transcriptional regulator
TM1059	-1.3	12.2	SpoVS-related protein
TM1063	-1.0	8.7	Oligopeptide ABC transporter
TM1064	-1.1	7.7	Oligopeptide ABC transporter
TM1067	-1.1	15.9	Oligopeptide ABC transporter
TM1072	-1.3	7.7	Sugar-phosphate aldolase
TM1104	-1.1	12.2	Conserved hypothetical

TM1105	-1.1	10.4	NADH dehydrogenase, putative
TM1112	-1.8	15.8	Hypothetical protein
TM1113	-1.1	8.1	Hypothetical protein
TM1127	-1.2	8.2	Hypothetical protein
TM1128	-1.6	5.8	Ferritin
TM1159	-1.1	11.4	Hypothetical protein
TM1170	-1.1	12.7	ABC transporter, periplasmic
TM1205	-1.0	11.9	Conserved hypothetical
TM1214	-1.1	4.4	NADH dehydrogenase, putative
TM1227	-1.2	7.9	Endo-1,4-beta-mannosidase
TM1232	-1.0	8.2	Sugar ABC transporter
TM1307	-1.1	6.7	Hypothetical protein
TM1322	-1.1	16.5	Conserved hypothetical
TM1330	-1.1	11.8	LacI family transcription regulator
TM1389	-1.7	13.9	Ubiquinone/menaquinone protein
TM1432	-1.2	11.2	Conserved hypothetical
TM1551	-1.1	10.5	Conserved hypothetical
TM1641	-2.1	15.5	Dihydrofolate reductase
TM1700	-1.3	14.3	Hypothetical protein
TM1701	-1.3	14.9	Conserved hypothetical
TM1803	-1.2	11.4	DnaJ-related protein
TM1874	-2.5	15.4	Cold shock protein
TMrrnaA	-2.2	18.8	
TMrrnaA	-2.0	12.4	
TM0016	1.3	17.9	Pyruvate ferredoxin oxidoreductase
TM0017	1.3	12.8	Pyruvate ferredoxin oxidoreductase
TM0018	1.1	10.4	Pyruvate ferredoxin oxidoreductase
TM0025	1.3	14.7	Beta-glucosidase
TM0035	1.1	10.4	Hypothetical protein
TM0068	1.2	13.2	D-mannonate oxidoreductase
TM0109	1.0	9.3	Pyruvate formate lyase
TM0110	1.8	15.3	Transcriptional regulator
TM0121	1.2	9.5	Conserved hypothetical
TM0139	1.0	11.5	Phosphoribosylanthranilate isomerase
TM0140	1.3	13.0	Indole-3-glycerol phosphatase
TM0146	1.3	11.9	ATP-dependent Clp protease
TM0148	1.9	16.1	Glucosamine--fructose-6
TM0169	1.7	16.0	Conserved hypothetical
TM0170	1.1	14.9	Hypothetical protein
TM0171	1.1	15.3	Conserved hypothetical
TM0172	1.2	10.0	Adenosylhomocysteinase
TM0198	1.5	12.9	ATP-dependent Clp protease
TM0207	1.2	13.2	Conserved hypothetical
TM0208	3.3	20.9	Pyruvate kinase
TM0209	1.9	16.9	6-phosphofructokinase
TM0210	1.0	6.9	Hypothetical protein
TM0219	1.6	14.0	Flagellar export/assembly protein
TM0257	1.7	18.5	
TM0258	1.1	14.0	DNA topoisomerase
TM0264	1.4	11.4	16S pseudouridylate synthase
TM0272	1.0	11.4	Pyruvate,orthophosphate dikinase
TM0274	1.6	14.1	Acetate kinase
TM0275	1.8	14.5	Transcriptional regulator

TM0381	1.1	12.2	Dihydrolipoamide dehydrogenase
TM0392	1.3	9.9	Conserved hypothetical
TM0395	1.8	4.1	NADH oxidase, putative
TM0396	1.4	16.5	Iron-sulfur cluster-binding protein
TM0427	1.0	14.7	Oxidoreductase, putative
TM0429	1.6	15.8	Methyl-accepting chemotaxis protein
TM0453	1.6	15.4	N utilization substance
TM0454	2.0	20.9	Ribosomal protein L11
TM0455	2.9	21.2	Ribosomal protein L1
TM0462	1.1	11.5	Conserved hypothetical
TM0463	1.6	14.4	Lipoprotein signal peptidase
TM0465	1.6	15.3	Hypothetical protein
TM0466	1.6	16.4	Conserved hypothetical
TM0467	1.3	9.7	Regulatory protein, putative
TM0477	1.0	4.1	Outer membrane protein
TM0505	1.3	15.5	GroES protein
TM0506	2.0	17.2	GroEL protein
TM0522	1.5	14.7	Heat shock protein HslU
TM0523	1.1	13.8	Hypothetical protein
TM0549	1.7	13.0	Acetolactate synthase
TM0604	1.0	16.2	Single stranded DNA-binding protein
TM0627	1.2	8.3	Lipopolysaccharide biosynthesis
TM0630	1.2	10.7	Nucleotide sugar epimerase
TM0631	1.1	13.8	Lipopolysaccharide biosynthesis
TM0687	1.7	14.3	Conserved hypothetical
TM0689	1.6	13.4	Phosphoglycerate kinase
TM0690	1.7	11.7	Conserved hypothetical
TM0693	2.2	18.8	Hypothetical protein
TM0694	2.5	17.5	Trigger factor, putative
TM0695	1.6	15.8	ATP-dependent Clp protease
TM0700	1.1	12.1	Chemotaxis response regulator
TM0701	1.4	12.6	Purine-binding chemotaxis
TM0702	3.0	6.0	Chemotaxis sensor histidine kinase
TM0703	1.1	9.8	Competence-damage inducible protein
TM0714	1.0	13.1	Hypothetical protein
TM0717	1.6	11.1	Propionyl-CoA carboxylase
TM0731	1.0	12.3	Conserved hypothetical
TM0744	1.2	13.8	Conserved hypothetical
TM0756	1.2	11.7	Galactosyltransferase
TM0758	1.2	12.9	Flagellin
TM0762	1.4	13.0	Ribosomal protein S2
TM0769	1.3	12.7	Phosphomannomutase
TM0772	1.1	9.1	Conserved hypothetical
TM0775	1.1	12.9	Translation initiation
TM0820	1.5	10.7	NADH-dependent butanol
TM0830	1.5	15.6	Conserved hypothetical
TM0836	1.1	17.5	Cell division protein F
TM0842	1.5	17.0	Response regulator
TM0847	1.5	12.6	Conserved hypothetical
TM0872	1.1	12.6	Conserved hypothetical
TM0877	1.1	17.5	Enolase
TM0896	2.2	21.8	Galactose-1-phosphate uridyltransferase
TM0902	1.4	9.7	RNA polymerase sigma-28

TM0903	1.3	13.8	Chemotaxis methylation
TM0904	1.6	15.4	Chemotaxis protein CheC
TM0905	1.4	15.4	Hypothetical protein
TM0912	1.1	11.2	Basic membrane protein
TM0919	1.2	5.1	Conserved hypothetical
TM0965	1.4	16.7	Phosphoribosylaminoimidazol carboxylase
TM0980	2.1	5.2	Uncharacterized protein
TM0981	1.9	17.0	Uncharacterized ACR
TM0982	1.3	15.7	Conserved hypothetical
TM1082	1.5	15.1	LexA repressor
TM1083	1.7	17.4	Conserved hypothetical
TM1148	1.2	13.4	Isocitrate dehydrogenase
TM1164	1.0	6.3	2-oxoacid ferredoxin oxidoreductase
TM1168	1.6	17.9	
TM1219	1.3	12.3	Oligopeptide ABC transporter
TM1220	1.2	15.4	Oligopeptide ABC transporter
TM1345	1.8	15.6	Polynucleotide phosphorylase
TM1346	1.3	12.1	Processing protease, putative
TM1347	1.5	11.7	Inosine-5'-monophosphatase
TM1360	2.0	18.5	Response regulator
TM1361	2.1	15.0	Isoleucyl-tRNA synthetase
TM1362	1.6	14.2	Motility protein PilT
TM1363	2.5	13.7	Peptide chain release factor
TM1364	1.6	15.2	Flagellar basal-body rod
TM1380	1.5	12.8	Conserved hypothetical
TM1381	1.9	16.7	Hypothetical protein
TM1382	1.6	17.8	Conserved hypothetical
TM1383	1.2	11.0	Conserved hypothetical
TM1391	1.1	10.5	ATP-dependent Clp protease
TM1399	1.0	14.5	Ribosome recycling factor
TM1400	2.0	13.3	Aspartate aminotransferase
TM1401	1.1	2.2	D-3-phosphoglycerate dehydrogenase
TM1420	1.0	16.2	Hypothetical protein
TM1421	1.0	14.4	Hydrogenase, putative
TM1422	1.7	15.1	RnfB-related protein
TM1425	1.0	15.1	Fe-hydrogenase, subunit
TM1426	1.4	20.2	Fe-hydrogenase, subunit
TM1437	1.1	9.2	Dimethyladenosine transferase
TM1438	1.8	13.5	
TM1439	1.5	14.1	Hypothetical protein
TM1442	1.8	14.2	Anti-sigma factor antagonist
TM1443	1.9	18.6	Cytidylate kinase
TM1444	1.1	11.8	LytB protein
TM1445	1.6	19.2	
TM1447	1.1	13.5	Conserved hypothetical
TM1451	1.6	12.3	RNA polymerase sigma-A
TM1453	1.1	10.5	Ribosomal protein S9
TM1454	1.2	12.0	Ribosomal protein L13
TM1455	1.0	9.0	Conserved hypothetical
TM1457	1.0	7.4	Hypothetical protein
TM1460	1.7	14.4	Jag protein, putative
TM1468	1.2	9.8	Conserved hypothetical
TM1469	1.0	10.5	Glucokinase

TM1470	1.6	15.6	Transcription terminate
TM1471	1.6	13.9	Ribosomal protein L17
TM1472	1.6	16.6	DNA-directed RNA polymer
TM1473	3.4	21.0	Ribosomal protein S4
TM1474	2.5	16.4	Ribosomal protein S11
TM1475	2.6	17.0	Ribosomal protein S13
TM1476	1.2	12.5	Ribosomal protein L36
TM1477	1.6	13.3	
TM1478	1.7	14.9	Methionine aminopeptidase
TM1479	2.3	18.7	Adenylate kinase
TM1482	2.1	17.5	Ribosomal protein L30
TM1483	2.3	14.9	Ribosomal protein S5
TM1484	1.4	2.8	Ribosomal protein L18
TM1485	3.1	11.8	Ribosomal protein L6
TM1486	2.9	21.2	Ribosomal protein S8
TM1487	1.5	12.1	Ribosomal protein S14
TM1488	3.0	19.9	Ribosomal protein L5
TM1489	2.1	3.7	Ribosomal protein L24
TM1490	1.8	13.3	Ribosomal protein L14
TM1491	2.6	20.0	Ribosomal protein S17
TM1492	1.9	21.0	Ribosomal protein L29
TM1493	2.0	14.5	Ribosomal protein L16
TM1494	2.8	19.7	Ribosomal protein S3
TM1495	2.5	4.1	Ribosomal protein L22
TM1497	1.8	18.7	Ribosomal protein L2
TM1498	2.6	22.8	Ribosomal protein L23
TM1499	2.6	22.7	Ribosomal protein L4
TM1500	2.4	16.5	Ribosomal protein L3
TM1501	1.5	15.3	Ribosomal protein S10
TM1502	2.3	17.9	Translation elongation
TM1503	1.8	19.0	Translation elongation
TM1504	2.3	19.7	Ribosomal protein S7
TM1509	1.1	9.5	Conserved hypothetical
TM1510	1.1	3.3	Hypothetical protein
TM1543	1.1	15.9	Flagellar basal-body rod
TM1545	1.2	14.4	Predicted endonuclease
TM1565	1.5	17.4	Signal recognition part
TM1566	1.3	10.4	Ribosomal protein S16
TM1568	1.7	16.6	16S rRNA processing protein
TM1569	2.6	20.1	tRNA guanine-N1 methyltransferase
TM1570	1.2	15.7	Conserved hypothetical
TM1571	1.3	11.8	Ribosomal protein L19
TM1572	1.7	3.7	Signal peptidase I, putative
TM1590	1.8	12.2	Translation initiation
TM1591	1.9	13.8	Ribosomal protein L35
TM1592	1.5	10.7	Ribosomal protein L20
TM1593	1.3	13.4	Conserved hypothetical
TM1605	2.4	19.8	Translation elongation
TM1606	1.8	20.4	Cytoplasmic axial filament
TM1607	1.9	15.0	Conserved hypothetical
TM1608	2.6	21.4	Conserved hypothetical
TM1611	1.4	10.1	ATP synthase F1, subunit
TM1613	1.6	18.7	ATP synthase F1, subunit

TM1621	1.1	1.8	hypothetical protein
TM1626	1.9	16.4	Peptidyl-tRNA hydrolase
TM1627	1.4	12.6	General stress protein
TM1628	1.1	15.7	Phosphoribosyl pyrophosphatase
TM1629	1.0	14.6	UDP-N-acetylglucosamine
TM1630	1.1	11.4	Hypothetical protein
TM1633	1.8	15.6	ATP-dependent protease
TM1654	1.7	13.9	Sensor histidine kinase
TM1684	1.5	10.1	Ribosomal protein L31
TM1685	1.1	11.2	Conserved hypothetical
TM1689	1.0	13.5	Guanylate kinase
TM1695	1.2	14.8	Conserved hypothetical
TM1704	1.1	10.3	Hypothetical protein
TM1706	2.0	17.4	Transcription elongation
TM1707	1.4	14.5	Conserved hypothetical
TM1710	1.1	11.2	
TM1763	1.5	13.7	Translation elongation
TM1764	1.2	2.0	Conserved hypothetical
TM1765	1.8	15.0	N utilization substance
TM1768	1.2	12.6	Exo-deoxyribonuclease VI
TM1777	1.0	11.7	N utilization substance
TM1809	1.6	13.8	Conserved hypothetical
TM1813	1.1	13.5	Hypothetical protein
TM1814	1.4	15.3	Conserved hypothetical
TM1858	1.3	13.5	RecX protein, putative
TM1859	1.0	13.6	DNA repair protein
TM1871	1.2	9.7	Hypothetical protein

(*) Original annotation (57)

TABLE 1.12 Differentially expressed ORFs for *P. furiosus* on co-culture LPM and pure culture *T. maritima* spent LPM at low cell density

Gene	Log (2) fold change CO vs. PST	-log(10)p-value	Annotation (*)
PF0199	1.1	12.5	Phosphoribosylformylglycinamidine (FGAM) synthase, synthetase domain
PF0421	1.2	14.9	ATP-utilizing enzymes of ATP-grasp superfamily
PF0422	1.0	13.6	Phosphoribosylamine-glycine ligase
PF0430	1.5	17.9	Formate-dependent phosphoribosylglycinamide formyltransferase
PF0677	1.4	12.2	Superfamily II helicase
PF0702	1.1	11.1	Xaa-Pro aminopeptidase
PF0729	1.0	8.5	Ferredoxin
PF0730	1.3	8.5	
PF0754	1.1	17.1	Pyruvate:ferredoxin oxidoreductase and related2-oxoacid:ferredoxin
PF0755	1.2	22.2	NAD-dependent aldehyde dehydrogenases
PF1088	1.3	18.4	Transcriptional regulators
PF1089	1.1	14.2	Permeases of the major facilitator
PF1285	2.3	20.6	ABC-type transport system
PF1286	1.4	13.7	ABC-type transport system
PF1461	1.1	11.2	L-asparaginase/archaeal Glu-tRNAGln amidotransferase subunit D
PF1530	1.0	10.7	Flavoprotein involved in thiazol biosynthesis
PF1658	1.1	16.3	ATP phosphoribosyltransferase
PF1659	1.7	13.6	Histidinol dehydrogenase
PF1660	1.5	21.1	Imidazoleglycerol-phosphate dehydratase
PF1661	1.5	14.1	Glutamine amidotransferase
PF1663A	1.0	15.1	
PF1664	1.1	14.1	Phosphoribosyl-AMP cyclohydrolase
PF1055	-1.8	22.7	Threonine synthase
PF1056	-1.8	22.5	Aspartate-semialdehyde dehydrogenase
PF0598	-1.7	20.9	Aspartate carbamoyltransferase, regulatory subunit
PF1241	-1.5	18.5	Uracil phosphoribosyltransferase
PF0599	-1.4	21.0	Aspartate carbamoyltransferase, catalytic chain
PF1054	-1.4	17.0	Homoserine kinase
PF1240	-1.4	18.4	Xanthine/uracil permeases
PF1053A	-1.4	14.4	
PF1053B	-1.4	13.2	
PF0163	-1.4	17.1	Predicted membrane protein
PF2032	-1.3	13.6	Na+/H+ antiporter

(*) Annotation for *P. furiosus* genes refers to the functional role assigned to their respective COGs (76).

TABLE 1.13. Differentially expressed ORFs for *P. furiosus* co-culture and pure culture on LPM at low cell density

Gene	Log (2) fold change CO vs. PFU	-log(10)p-value	Annotation (*)
PF0287	1.6	17.9	Subtilisin-like serine proteases
PF0346	1.1	8.0	Aldehyde:ferredoxin oxidoreductase
PF0430	1.1	5.5	Formate-dependent phosphoribosylglycinamide formyltransferase
PF0442	1.0	13.2	Beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase
PF0551	1.0	3.3	
PF0661	1.2	1.3	Predicted transcriptional regulators
PF0677	1.6	0.8	Superfamily II helicase
PF0688	1.1	1.8	Subtilisin-like serine proteases
PF0695	1.1	8.5	Uncharacterized conserved protein
PF0702	1.1	0.1	Xaa-Pro aminopeptidase
PF0706	1.1	10.9	Uncharacterized protein conserved in archaea
PF0718	1.3	14.2	
PF0721	1.0	13.1	Predicted flavoprotein
PF0722	1.3	17.9	Peroxiredoxin
PF0729	1.1	0.1	Ferredoxin
PF0730	1.4	0.5	
PF0747	1.0	19.9	Xaa-Pro aminopeptidase
PF0753	1.0	3.7	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF0754	1.4	3.8	Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin
PF0755	1.4	5.0	NAD-dependent aldehyde dehydrogenases
PF1006	1.0	1.2	ABC-type phosphate transport system, permease
PF1203	1.0	22.5	Aldehyde:ferredoxin oxidoreductase
PF1253	1.0	11.4	Aspartate/tyrosine/aromatic aminotransferase
PF1854	1.1	1.6	Molybdenum cofactor biosynthesis enzyme
PF2061	1.2	1.1	ABC-type multidrug transport system, permease
PF0200	-1.2	21.3	Uncharacterized conserved protein
PF0201	-1.3	19.9	Aconitase A
PF0202	-1.4	19.2	Isocitrate dehydrogenases
PF0203	-1.4	16.5	Citrate synthase
PF0488	-1.1	5.1	Ribosomal protein S6E (S10)
PF0934	-1.4	18.0	Superfamily II DNA/RNA helicases, SNF2
PF0935	-1.0	13.2	Thiamine pyrophosphate-requiring enzymes acetolactate synthase
PF0936	-2.2	23.0	Ketol-acid reductoisomerase
PF0937	-1.7	20.3	Isopropylmalate/homocitrate/citramalate synthases
PF0938	-1.5	19.6	3-isopropylmalate dehydratase large subunit
PF0939	-1.2	16.3	3-isopropylmalate dehydratase small subunit
PF0940	-1.5	23.2	Isocitrate/isopropylmalate dehydrogenase
PF0941	-1.7	15.5	Isopropylmalate/homocitrate/citramalate synthases

PF0942	-1.6	20.7	Dihydroxyaciddehydratase/phosphogluconate dehydratase
PF1032	-1.2	14.9	NMD protein affecting ribosome stability
PF1053A	-1.8	4.9	
PF1053B	-1.7	3.2	
PF1054	-1.5	0.6	Homoserine kinase
PF1055	-1.5	5.5	Threonine synthase
PF1056	-1.8	0.0	Aspartate-semialdehyde dehydrogenase
PF1062	-1.1	11.7	Predicted RNA-binding protein
PF1109	-1.1	17.9	
PF1110	-1.3	17.9	
PF1412	-1.7	4.3	ABC-type dipeptide/oligopeptide/nickel transport system, ATPase
PF1678	-2.3	27.1	Isopropylmalate/homocitrate/citramalate synthases
PF1679	-2.4	20.1	3-isopropylmalate dehydratase large subunit
PF1680	-1.6	22.3	3-isopropylmalate dehydratase small subunit
PF1683	-1.3	17.7	Acetylglutamate semialdehyde dehydrogenase
PF1684	-1.1	14.1	Acetylglutamate kinase
PF1685	-1.3	13.4	4-aminobutyrate aminotransferase and relatedaminotransferases
PF1686	-1.2	11.6	Acetylornithine deacetylase/Succinyl-diaminopimelate desuccinylase
PF1702	-1.0	13.2	Aspartate/tyrosine/aromatic aminotransferase
PF1739	-3.2	26.5	ABC-type sugar transport system, periplasmic
PF1742	-2.4	16.0	Glycosyltransferase
PF1743	-1.9	21.2	Predicted transcriptional regulators
PF1744	-1.2	13.1	ABC-type sugar transport systems, ATPase
PF1881	-1.2	7.3	Archaeal DNA-binding protein
PF1933	-1.2	15.6	ABC-type sugar transport systems, ATPase
PF1934	-1.4	18.1	
PF1935	-1.2	17.2	Alpha-amylase/alpha-mannosidase
PF1936	-1.2	17.6	ABC-type sugar transport system, permease
PF1938	-1.3	13.5	Maltose-binding periplasmic proteins/domains
PF1951	-1.1	15.1	Aspartyl/asparaginyl-tRNA synthetases

(*) Annotation for *P. furiosus* genes refers to the functional role assigned to their respective COGs (76).

TABLE 1.14. Differentially expressed ORFs for *P. furiosus* on PST-LPM and pure culture LPM at low cell densities

Gene	Log (2) fold change PST vs. PFU	-log(10)p-value	Annotation (*)
PF0074	1.1	13.9	Dehydrogenases with different specificities
PF0075	1.1	9.9	Alcohol dehydrogenase, class IV
PF0121	1.3	16.9	Transcriptional regulators containing a DNA-binding domain
PF0163	1.6	18.6	Predicted membrane protein
PF0193	1.1	18.7	ABC-type dipeptide/oligopeptide/nickel transport system, ATPase
PF0287	2.3	17.9	Subtilisin-like serine proteases
PF0344	1.0	11.7	Predicted Fe-S oxidoreductases
PF0345	1.1	13.6	Molybdopterin converting factor, small subunit
PF0346	1.7	8.0	Aldehyde:ferredoxin oxidoreductase
PF0442	1.0	13.2	Beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase
PF0533	1.1	13.9	Pyruvate:ferredoxin oxidoreductase and related2-oxoacid:ferredoxin
PF0547	1.0	9.3	Predicted GTPase
PF0556	1.3	11.3	
PF0598	1.5	19.4	Aspartate carbamoyltransferase, regulatory subunit
PF0599	1.3	19.6	Aspartate carbamoyltransferase, catalytic chain
PF0706	1.1	10.9	Uncharacterized protein conserved in archaea
PF0707	1.1	11.4	
PF0709	1.3	14.5	Transcriptional regulators
PF0716	1.3	14.0	3-hydroxyisobutyrate dehydrogenase and related beta-hydroxyacid
PF0718	1.6	14.2	
PF0721	1.0	13.1	Predicted flavoprotein
PF0722	1.2	17.9	Peroxiredoxin
PF0736	1.0	9.7	
PF0742	1.2	15.0	Ferritin-like protein
PF0747	1.3	19.9	Xaa-Pro aminopeptidase
PF0765	1.0	18.8	UDP-N-acetyl-D-mannosaminuronate dehydrogenase
PF0891	1.0	14.4	Ferredoxin
PF1196	1.4	20.3	Uncharacterized conserved protein
PF1203	1.8	22.5	Aldehyde:ferredoxin oxidoreductase
PF1240	1.8	21.1	Xanthine/uracil permeases
PF1241	1.8	20.7	Uracil phosphoribosyltransferase
PF1253	1.0	11.4	Aspartate/tyrosine/aromatic aminotransferase
PF1411	1.1	12.0	ABC-type dipeptide/oligopeptide/nickel transport system, ATPase
PF1421	1.1	14.8	4-aminobutyrate aminotransferase and relatedaminotransferases

PF1423	1.1	11.1	Multisubunit Na+/H+ antiporter, MnhE subunit
PF1425	1.2	17.2	Multisubunit Na+/H+ antiporter, MnhG subunit
PF1426	1.1	16.6	
PF1429	1.1	16.2	Multisubunit Na+/H+ antiporter, MnhC subunit
PF1456	1.1	15.3	Histone acetyltransferase HPA2 andrelated
PF1497	1.1	13.9	Aspartate/tyrosine/aromatic aminotransferase
PF1532	1.6	20.2	Uncharacterized NAD(FAD)-dependent dehydrogenases
PF1547	1.1	12.3	Cellulase M and related proteins
PF1549	1.4	14.7	RNA 3'-terminal phosphate cyclase
PF1563	1.4	14.7	DNA-directed RNA polymerase, beta subunit/160
PF1564	1.4	19.2	DNA-directed RNA polymerase, beta subunit/140
PF1573	1.3	13.8	Predicted Zn-dependent proteases
PF1696	1.1	12.9	ABC-type uncharacterized transport systems, ATPase
PF1719	1.1	14.9	Putative intracellular protease/amidase
PF1778	1.4	15.7	Glycine/serine hydroxymethyltransferase
PF1920	1.3	13.3	Triosephosphate isomerase
PF1999	1.1	14.9	Glycine cleavage system protein P
PF2000	1.1	12.5	Glycine cleavage system protein P
PF0126	-1.1	10.5	DNA or RNA helicase
PF0132	-1.1	13.9	
PF0133	-1.2	10.9	Uncharacterized conserved protein
PF0200	-1.8	21.3	Uncharacterized conserved protein
PF0201	-1.7	19.9	Aconitase A
PF0202	-1.6	19.2	Isocitrate dehydrogenases
PF0203	-2.0	16.5	Citrate synthase
PF0204	-1.2	12.5	Glutamate synthase domain 1
PF0450	-1.4	18.2	Glutamine synthetase
PF0934	-1.4	18.0	Superfamily II DNA/RNA helicases, SNF2
PF0935	-1.3	13.2	Thiamine pyrophosphate-requiring enzymes
PF0936	-3.1	23.0	Ketol-acid reductoisomerase
PF0937	-2.3	20.3	Isopropylmalate/homocitrate/malate synthases
PF0938	-2.0	19.6	3-isopropylmalate dehydratase large subunit
PF0939	-1.5	16.3	3-isopropylmalate dehydratase small subunit
PF0940	-1.9	23.2	Isocitrate/isopropylmalate dehydrogenase
PF0941	-2.0	15.5	Isopropylmalate/homocitrate/citramalate synthases
PF0942	-2.1	20.7	Dihydroxyaciddehydratase/phosphogluconate dehydratase
PF1032	-1.5	14.9	NMD protein affecting ribosome stability
PF1088	-1.0	15.3	Transcriptional regulators
PF1109	-1.8	17.9	
PF1110	-2.1	17.9	
PF1284	-1.3	15.8	
PF1285	-2.1	19.4	ABC-type transport system
PF1286	-1.2	12.1	ABC-type transport system n
PF1461	-1.0	10.7	L-asparaginase/archaeal Glu-tRNA Gln amidotransferase subunit D
PF1657	-1.1	12.0	Histidyl-tRNA synthetase

PF1658	-1.1	16.1	ATP phosphoribosyltransferase
PF1659	-1.7	13.6	Histidinol dehydrogenase
PF1660	-1.5	20.8	Imidazoleglycerol-phosphate dehydratase
PF1661	-1.7	15.2	Glutamine amidotransferase
PF1662	-1.3	17.5	Phosphoribosylformimino-5-aminoimidazole carboxamide ribonucleotide
PF1663A	-1.4	18.1	
PF1663B	-1.6	13.5	
PF1664	-1.4	16.6	Phosphoribosyl-AMP cyclohydrolase
PF1678	-2.6	27.1	Isopropylmalate/homocitrate/citramalate synthases
PF1679	-2.7	20.1	3-isopropylmalate dehydratase large subunit
PF1680	-2.1	22.3	3-isopropylmalate dehydratase small subunit
PF1683	-1.4	17.7	Acetylglutamate semialdehyde dehydrogenase
PF1684	-1.2	14.1	Acetylglutamate kinase
PF1685	-1.4	13.4	4-aminobutyrate aminotransferase and related aminotransferases
PF1686	-1.2	11.6	Acetylornithine deacetylase/Succinyl-diaminopimelate desuccinylase
PF1702	-1.2	13.2	Aspartate/tyrosine/aromatic aminotransferase
PF1704	-1.2	18.0	
PF1739	-2.8	26.5	ABC-type sugar transport system,periplasmic
PF1742	-2.5	16.0	Glycosyltransferase
PF1743	-2.0	21.2	Predicted transcriptional regulators
PF1744	-1.1	13.1	ABC-type sugar transport systems, ATPase
PF1786	-1.2	11.9	
PF1933	-1.2	15.6	ABC-type sugar transport systems, ATPase
PF1934	-1.8	18.1	
PF1935	-1.5	17.2	Alpha-amylase/alpha-mannosidase
PF1936	-1.5	17.6	ABC-type sugar transport system,permease
PF1938	-1.8	13.5	Maltose-binding periplasmic proteins/domains
PF1951	-1.3	15.1	Aspartyl/asparaginyl-tRNA synthetases

(*) Annotation for *P. furiosus* genes refers to the functional role assigned to their respective COGs (76).

TABLE 1.15. Differentially expressed ORFs for *T. maritima* co-culture on LPM and *P. furiosus* spent LPM at low cell densities

Gene	Log (2) fold change CO vs. TSP	-log(10)p-value	Annotation (*)
TM0373	1.6	15.5	DNA K
TM0381	1.3	11.3	Dihydrolipoamide dehydrogenase
TM0576	1.1	11.5	DNA polymerase III, alpha subunit
TM0850	1.0	9.2	GrpE protein, putative
TM0980	1.2	10.6	Uncharacterized protein
TM0981	1.5	13.0	Uncharacterized ACR
TM0982	1.4	13.6	Conserved hypothetical
TM0983	1.5	11.8	Conserved hypothetical
TM1375	1.3	11.0	Spermidine/putrescine A
TM1839	1.2	6.4	Maltose ABC transporter
TM_rnpB	-1.8	11.9	
TM0054	-1.0	9.6	Hypothetical protein
TM0144	-1.5	10.6	Conserved hypothetical
TM0150	-1.0	10.9	Ribosomal protein L32
TM0257	-1.1	9.9	
TM0266	-1.3	16.7	DNA-binding protein, HU
TM0394	-1.3	13.3	Conserved hypothetical
TM0395	-1.2	11.2	NADH oxidase, putative
TM0451	-2.2	21.3	Ribosomal protein L33
TM0544	-1.3	14.2	ABC transporter, ATP-bi
TM0545	-1.3	14.2	Homoserine kinase, putative
TM0546	-1.2	12.0	Threonine synthase
TM0549	-1.3	11.1	Acetolactate synthase
TM0550	-1.4	7.9	Ketol-acid reductoisomerase
TM0551	-1.3	7.0	Dihydroxy-acid dehydratase
TM0552	-1.3	8.2	2-isopropylmalate synthase
TM0553	-1.5	9.3	2-isopropylmalate synthase
TM0554	-1.5	14.0	3-isopropylmalate dehydratase
TM0555	-1.8	18.1	3-isopropylmalate dehydydratas
TM0556	-1.6	13.3	3-isopropylmalate dehydratase
TM0758	-1.0	7.4	Flagellin
TM0927	-1.1	11.1	Ferredoxin
TM1146	-1.5	13.8	Methyl-accepting chemotaxis protein
TM1147	-2.0	14.4	Conserved hypothetical
TM1246	-1.4	12.3	Phosphoribosylformylglycinamide synthase
TM1251	-1.2	11.1	Phosphoribosylformylglycinamide cyclo-ligase
TM1300	-1.0	10.1	Hypothetical protein
TM1303	-1.8	15.8	Conserved hypothetical
TM1304	-1.1	4.2	Conserved hypothetical
TM1316	-4.2	23.5	Hypothetical protein
TM1317	-2.0	21.0	AtsB/chuR-related protein
TM1318	-2.0	16.1	

TM1319	-2.1	14.3	ABC transporter, ATP binding protein
TM1323	-1.5	13.5	Hypothetical protein
TM1328	-2.1	9.5	ABC transporter, ATP-binding protein
TM1334	-2.4	15.9	Conserved hypothetical
TM1335	-2.9	17.6	Hypothetical protein
TM1336	-2.9	19.0	Permease, putative
TM1347	-1.5	13.9	Inosine-5'-monophosphatase
TM1374	-1.6	11.3	Phosphoglycerate mutase
TM1657	-1.1	7.4	Ribosomal protein S20
TM1683	-1.5	12.8	Cold shock protein
TM1786	-1.9	17.7	Hypothetical protein

(*) Original annotation (57)

TABLE 1.16. Differentially expressed ORFs for *T. maritima* co-culture and pure culture in LPM at low cell density

Gene	Log (2) fold change CO vs. TMA	-log(10)p-value	Annotation (*)
TM0373	1.4	14.4	DnaK protein
TM0505	1.0	10.3	GroES protein
TM0978	1.1	12.6	Conserved hypothetical
TM0980	1.6	13.8	Uncharacterized protein
TM0981	2.0	16.2	Uncharacterized ACR
TM0982	2.7	20.8	Conserved hypothetical
TM0983	2.2	15.9	Conserved hypothetical
TM1223	1.5	15.8	Oligopeptide ABC transporter
TM1375	1.2	10.6	Spermidine/putrescine A
TM1483	1.0	8.7	Ribosomal protein S5
TM1484	1.0	8.2	Ribosomal protein L18
TM1486	1.1	9.6	Ribosomal protein S8
TM1491	1.0	12.0	Ribosomal protein S17
TM1492	1.1	8.6	Ribosomal protein L29
TM1498	1.2	11.9	Ribosomal protein L23
TM1500	1.2	10.1	Ribosomal protein L3
TM_rnpB	-1.6	11.2	
TM_TMrr	-2.1	15.5	
TM0044	-1.1	8.2	Hypothetical protein
TM0144	-1.2	8.5	Conserved hypothetical
TM0150	-1.0	11.1	Ribosomal protein L32
TM0192	-1.5	15.9	SpoVS-related protein
TM0255	-1.4	9.8	Ribosomal protein L28
TM0266	-1.8	20.3	DNA-binding protein, HU
TM0451	-1.0	12.8	Ribosomal protein L33
TM0504	-2.4	16.4	Hypothetical protein
TM0606	-1.0	9.0	Hypothetical protein
TM0662	-1.4	12.7	Acyl carrier protein
TM0927	-1.7	15.9	Ferredoxin
TM1127	-1.1	10.9	Hypothetical protein
TM1128	-1.1	12.0	Ferritin
TM1147	-1.1	8.3	Conserved hypothetical
TM1374	-1.7	11.7	Phosphoglycerate mutase
TM1657	-1.3	9.1	Ribosomal protein S20
TM1683	-1.3	11.2	Cold shock protein
TM1786	-1.1	11.8	Hypothetical protein
TM1850	-1.3	10.5	Hypothetical protein
TM1874	-1.0	8.9	Cold shock protein

(*) Original annotation (57)

TABLE 1.17. Differentially expressed ORFs for *T.maritima* pure culture in TSP-LSM and pure culture in LSM at low cell density

Gene	Log (2) fold change TSP vs. TMA	-log(10)p-value	Annotation (*)
TM0025	1.2	14.2	Beta-glucosidase
TM0031	1.1	6.5	Oligopeptide ABC transp
TM0451	1.2	14.5	Ribosomal protein L33
TM0546	1.2	12.1	Threonine synthase
TM0550	1.1	6.2	Ketol-acid reductoisome
TM0552	1.0	6.2	2-isopropylmalate synthase
TM0553	1.6	10.4	2-isopropylmalate synthase
TM0554	1.7	15.2	3-isopropylmalate dehydratase
TM0555	1.7	17.6	3-isopropylmalate dehydratase
TM0556	1.3	11.0	3-isopropylmalate dehydratase
TM0690	1.2	8.5	Conserved hypothetical
TM0903	1.0	13.3	Chemotaxis methylation
TM0982	1.3	12.8	Conserved hypothetical
TM1219	1.0	11.0	Oligopeptide ABC transporter
TM1223	1.2	13.9	Oligopeptide ABC transporter
TM1300	1.0	10.1	Hypothetical protein
TM1303	1.9	16.5	Conserved hypothetical
TM1304	1.2	5.0	Conserved hypothetical
TM1316	4.2	23.6	Hypothetical protein
TM1317	2.1	21.2	AtsB/chuR-related protein
TM1318	1.7	14.7	
TM1319	2.2	14.7	ABC transporter, ATP-binding protein
TM1323	1.2	10.9	Hypothetical protein
TM1328	2.1	9.6	ABC transporter, ATP-binding protein
TM1334	2.7	17.4	Conserved hypothetical
TM1335	3.3	19.0	Hypothetical protein
TM1336	3.1	19.8	Permease, putative
TM1347	1.4	12.8	Inosine-5'-monophosphatase
TM1363	1.2	12.9	Peptide chain release factor
TM1476	1.2	9.8	Ribosomal protein L36
TM1497	1.1	11.0	Ribosomal protein L2
TM1500	1.1	9.4	Ribosomal protein L3
TM1503	1.2	8.6	Translation elongation
TM1611	1.0	12.5	ATP synthase F1, subunit
TM0255	-1.0	7.2	Ribosomal protein L28
TM0504	-2.4	16.8	Hypothetical protein
TM0575	-1.1	8.9	Crossover junction endonuclease
TM1125	-1.1	6.7	Hypothetical protein
TM_rRNA 5S	-1.9	14.6	rRNA 5S.

(*) Original annotation (57)

CHAPTER 2:

Response of wild-type and resistant-mutant strains of the hyperthermophilic bacterium *Thermotoga maritima* to chloramphenicol challenge

Clemente I. Montero, Matthew R. Johnson, Chung-Jung Chou, Shannon B. Conners, Sarah G. Geouge, Sabrina Tachdjian, Jason D. Nichols and Robert M. Kelly

Department of Chemical and Biomolecular Engineering
North Carolina State University
Raleigh, NC 27695-7905

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ABSTRACT

A whole genome cDNA microarray was used to interrogate the time-dependent transcriptional response of both wild-type (WT) and resistant mutant (RM) strains of the hyperthermophilic bacterium *Thermotoga maritima* to the translation inhibiting antibiotic chloramphenicol (CAM). There were substantial differences between the transcriptomes of the two strains that reflected metabolic perturbations and responses attributed to antibiotic sensitivity and resistance. In batch culture (80°C, 10^7 cells/ml), the WT strain exhibited significant morphological changes within 30 minutes following a 100 μg/ml pulse, while no such changes were noted for the RM. In chemostat culture (80°C, 10^8 cells/ml, dilution rate of 0.42 h^{-1}), continuous addition of CAM at 100 μg/ml final concentration led to washout of the WT, while having no noticeable effect on the RM. Analysis of time-dependent differential gene expression for batch and continuous cultures revealed substantial differences between the transcriptomes of the two strains, both before and after CAM challenge. For the WT, CAM response was immediate, resembling previously described transcriptional profiles reported for mesophilic bacteria responding to CAM. In both batch and continuous culture, the WT CAM response indicated up-regulation of a polyamine synthetic pathway, as well as an oxidative stress response, possibly related to a reactive oxygen detoxification process observed in the hyperthermophilic archaeon *Pyrococcus furiosus*. The RM transcriptome indicated a CAM response, but after a significant delay compared to the WT. Differences between the WT and RM prior to CAM challenge suggested that the RM was pre-conditioned to antibiotic exposure and mechanisms to minimize impact were already operational (up-regulation of ribosomal proteins, cold shock proteins, polyamine synthesis). The basis for

RM insensitivity to CAM was found to be, at least in part, attributable to 5 mutations in its 23S rRNA, two of which (G2447A and G2057A) were located at the peptidyltransferase center (PTC). In addition to providing information on the sensitivity/response/resistance of a primitive bacterium to antibiotic challenge, this study reinforces the use of functional genomics tools to investigate antibiotic adaptation mechanisms. Exploring antibiotic resistance and adaptation in hyperthermophiles could provide unique complementary insights that would be useful in formulating therapeutic strategies.

INTRODUCTION

Since their introduction into medical practice in the 1940s, antibiotics have had a significant impact on microbial populations, modifying their diversity, physiology, and evolution. In response to antibiotic-based selective pressures, microorganisms have developed genetic and biochemical resistance strategies to the point that certain modern bacterial infections have become almost untreatable. Some antibiotic resistance, however, appears to have developed independently of human intervention; phylogenetic analysis has shown that some antibiotic resistance genes evolved on plasmids millions of years ago, and have since been exchanged among bacteria (10, 11). As such, it has been proposed that antibiotics have impacted evolutionary relationships among prokaryotes (43).

The genomics era has given rise to unprecedented opportunities for obtaining new insights that could result in targeted therapeutic strategies to combat emerging antibiotic resistant microorganisms (37). Previous studies have shown that genome-wide transcriptional profile information on antibiotic exposure can help identify reactive or resistance mechanisms for a particular microorganism being challenged (18, 37, 54, 79, 85). In some cases, response to antibiotic response is highly variable from one microorganism to another (19, 40). In other instances, for antibiotics targeting translational inhibition, surprisingly similar features in the transcriptional response have been reported for phylogenetically diverse groups of mesophilic bacteria (18, 85, 101). For example, the response to natural antibacterial agents, such as chloramphenicol (CAM), a competitive inhibitor of the peptidyl transfer reaction catalyzed by the 23S rRNA (123), is usually associated with the up-regulation of genes related to translational

machinery and the purine biosynthetic pathway along with down-regulation of amino acid biosynthetic genes and aminoacyl-tRNA synthases (18, 54, 85). In addition, exposure to CAM elicits physiological responses that can individually or collectively induce bacteriostatic or bactericidal effects (102). These include increased translation inaccuracy events *in vivo* (111), filamentation (105), cold shock response (115), transient oxidative response (2), stringent response (28), polyamine production (95, 96), and up-regulation of genes encoding ribosomal proteins (18, 85, 101). Mechanisms of resistance to CAM that have been observed include: modifications of 23S rRNA , attenuation by phosphorylation (56) or acetylation (103), hydrolysis to an inactive form (81), alterations of cell wall permeability (97), or by the activation of efflux pumps (88). Determining the contribution of specific elements to the overall antibiotic response is complicated by the fact that the actual mechanism of interference or resistance might be obscured by other phenomena (102, 113). However, the recent availability of a range of powerful genomics and molecular tools provide a means to tease apart confounding antibiotic exposure effects.

The mechanisms of antibiotic response and resistance in microorganisms have been mostly studied for a limited set of mesophilic bacteria, and not in microorganisms that inhabit biologically restrictive niches only minimally impacted by human intervention. *Thermotoga maritima*, is an anaerobic, hyperthermophilic bacterium (growth $T_{opt} \geq 80°C$) that was originally isolated from hydrothermal waters near Vulcano Island, Italy (52). According to 16S rRNA phylogenetic trees, this bacterium resides in a deeply-branched evolutionary position within the Bacteria (17). Genome sequence information indicates that *T. maritima* has apparently been involved in substantial

amounts of horizontal gene transfer with hyperthermophilic Archaea (84). Previous reports have demonstrated that, unlike other eubacteria, *T. maritima* is resistant to all known groups of aminoglycosides and utilizes a fusidic acid-insensitive, diphtheria toxin-resistant, elongation factor G (EF-G) (74). On the other hand, in common with less thermophilic bacteria, *T. maritima* was found to be sensitive to CAM (52), allowing for direct comparisons between the response mechanisms of this hyperthermophile and mesophilic bacteria. This was pursued further here using a whole genome cDNA microarray to probe the transcriptional response of wild-type *T. maritima* to CAM challenge. A CAM-resistant strain was isolated so that mechanisms of CAM resistance in this mutant could be probed and compared to those of model mesophilic bacteria. In addition to providing information on the sensitivity/response/resistance of a primitive bacterium to antibiotic challenge, this study reinforces the use of functional genomics tools for investigating antibiotic adaptation mechanisms. Exploring antibiotic resistance and adaptation in hyperthermophiles could provide unique complementary insights that would be useful in formulating therapeutic strategies (37).

MATERIALS AND METHODS

Growth of the microorganism in batch and continuous culture. *T. maritima* MSB8 was grown anaerobically on Sea Salts Medium (SSM), as previously described (93). Medium pH was adjusted to 6.8 with NaOH, and autoclaved prior to use. For a carbon source, a stock cellobiose (Sigma) solution was prepared by sterilization using 0.22 μm membrane filters (Millipore) and added to the SSM to a final concentration of 10 mM prior to inoculation. Batch cultures (50 ml) were inoculated under N_2 (high purity; National Welders) headspace, and were grown at 80°C for 8 to 12 hours in oil baths. Growth was monitored by epifluorescence microscopic cell density enumeration, as previously described (48, 58). For batch CAM challenge, a 16l Microgen sterilizable-in-place fermenter (New Brunswick Scientific, Edinson, NJ) was used; 8l of media were prepared by heating to 100°C for 20 min. Prior to inoculation, the medium was reduced with 40 ml of 10% (w/v) NaS. A 2% inoculum was added anaerobically through positive pressurization of culture bottles directly connected with Norprene A60F tubing (Saint-Gobain, Bridgewater, NJ) to the inoculation port of the fermenter. The culture was grown at 80°C and 200 RPM agitation with continuous sparging with N_2 to maintain anaerobic conditions.

Continuous cultivation of *T. maritima* was performed in a 2l five-neck, round-bottom flask, as described previously (93). A 60 ml batch culture was used to inoculate 2l of SSM supplemented with cellobiose to a final concentration of 10 mM. This seed culture was grown in the 2-L flask at 80°C for 7 hours under continuous nitrogen sparging, and continuous culture was then initiated by adding fresh media (made up in

12l stocks) at a dilution rate of 0.42 h^{-1}. Steady-state conditions were monitored by following direct cell counts as described previously (93)

In batch culture, CAM was added to a final concentration of 100 μg/ml. Wild-type (WT) and CAM-resistant (RM) strains were challenged during mid-exponential phase corresponding to a cell density of 2 x 10^7 cells/ml. Samples were then taken at time 0 (immediately before the CAM challenge), 5 and 30 min after the challenge. For continuous culture, CAM was added to the culture anaerobically, such that the culture was immediately exposed to 100 μg/ml; simultaneously, CAM was supplemented to the feed to 100 μg/ml such that the culture was continuously exposed to this level of the antibiotic.

For both batch and continuous culture, 350 ml of culture were harvested and cooled rapidly to 0°C by immersing centrifuge bottles containing the sample in ice water; these bottles were then immediately centrifuged at 10,000 x g for 15 min at 4°C. RNA extraction was then performed following protocols described previously (39).

Imaging and microscopy methods. Epifluorescent micrographs were taken with a SPOT digital camera attached to a microscope (Southern Micro Instruments) with 100X oil-immersion lens. Cell suspensions were fixed in 2.5% glutaraldehyde and stained with acridine orange (1 g/l; Fisher Scientific) to determine cell densities.

Isolation of a chloramphencol-resistant mutant and determination of Minimum Inhibitory Concentration (MIC). A CAM-resistant mutant strain of *T. maritima* MSB8, to be referred to here as strain RM, was selected by successive passages

in SSM with increasing concentrations of CAM (5 µg/ml to 500 µg/ml). The RM strain was isolated by serial dilution of the culture to extinction, as described by previously reported methods (12). MIC were determined by monitoring OD_{600} (in triplicate) and by direct cell counts, following the guidelines of NCCLS (59) MIC here was defined as the highest concentration of CAM that gave an $OD_{600} < 0.05$ for the culture when assayed after a 24 h incubation at 80°C on SSM. Antibiotic stability was tested in triplicates using an *Escherichia coli* bioassay, as described previously (89, 125); stock solutions of SSM + 4 mg/ml CAM were incubated at 25°C and 80°C; samples were taken at 0, 12, 24, 36 and 48 hours and frozen at -20°C. For each replicate, fresh LB media was inoculated with a 1:1000 dilution of an *E. coli* culture at OD_{600} of ~0.5-0.7 to a final cell density of between 1 to $5x10^6$ CFU/ml. These *E. coli* cultures were then used to perform 2-fold dilutions of the frozen stock solutions of SSM + CAM in 96 well plates. After a 24 hour incubation at 37°C, OD_{600}s were measured using a HTS 7000 Plus 96-well plate reader. (Perkin-Elmer).

There was only mild thermal deterioration of CAM under the experimental conditions tested in our bioassay. Even after 48 hours of at 78°C, ~60% of CAM activity remained, a result comparable to previous reports using *E. coli* bioassays in which the half-life of CAM at 72°C under anaerobic conditions was between 60 and 70 hours (89).

DNA sequencing. DNA sequencing of the full 23S rRNA (3.0 Kb) of the RM strain was performed by the Integrated Biotech Laboratories at University of Georgia (http://www.ssf.uga.edu/). Sequencing was performed both directly from genomic DNA and from a purified PCR product amplified by the primers rRNA1F 5'-

CCCTCGTCTCCACCAAGG-3'and rRNA1R 5'-GGGTCACTGGAAACTGCATAGG-3'.

Construction of the whole-genome cDNA microarray. Construction of the whole genome cDNA microarray of *T. maritima* has been described in detail elsewhere (24, 58, 93). PCR products were generated for all of the open reading frames from the *T. maritima* MSB8 genome, available at http://www.tigr.org/, purified, re-suspended and randomly distributed for printing onto Corning Gaps II slides. Six replicates for each gene were printed per slide using a Q-Array Mini printer (Genetix).

Labeling and hybridization. cDNA was generated from the extracted RNA and labeled using the indirect aminoallyl labeling method described elsewhere (46, 93) Hybridizations were performed for 18 hours at 42°C following protocols described previously (46, 47). Slides were analyzed using an ExpressLite Scanner and quantitated using the ScanArray 2.1 software (Perkin-Elmer).

Statistical analyses and determination of differential gene expression. Statistical analysis was performed as described previously (93). Replication of treatments, arrays, dyes, and cDNA spots allowed the use of analysis of variance (ANOVA) models using a loop design for reciprocal labeling hybridizations (64, 65). Briefly, a linear normalization ANOVA model (122) was used to estimate global variation in the form of fixed (dye, D; treatment, T) and random [array, A; spot, A(Bl)] effects and random error using the model $\log_2(y_{ijklmn}) = \mu + A_i + D_j + T_k + A_i(B_l) + \varepsilon_{ijklm}$.

A gene-specific ANOVA model was then used to partition the remaining variation into gene-specific effects using the model $r_{ijklm} = \mu + A_i + D_j + T_k + A_i(B_l) + \varepsilon_{ijklm}$. Unless otherwise noted, original gene annotations have been confirmed against the COG database at NCBI (108) and the Conserved Domain Database at NCBI (78). For information on the magnitude and statistical significance of fold changes for all of the genes included on the array, see http://www.che.ncsu.edu/extremophiles/.

RESULTS and DISCUSSION

Isolation of CAM-resistant mutant and determination of MIC. A CAM-resistant mutant (RM) of *T. maritima* MSB8 was isolated by successive passages in SSM concomitant with increasing concentrations of CAM (ranging from 5 μg/ml to 500 μg/ml), followed by serial dilution to extinction. The RM strain was found to have an MIC of ~1 mg/ml, significantly higher than the MIC for the WT, which was 25 μg/ml (see Figure 2.1). There was only a slight loss in efficacy of CAM at 80°C after 48 hours, as confirmed through *E. coli* bioassay (data not shown).

Growth physiology of WT and RM strains of *T. maritima* challenged with CAM. CAM (100 μg/ml) was added to exponentially growing batch cultures of the WT and RM strains as a pulse at the point when cell densities reached approximately 10^7 cells/ml; this corresponded to 10 pg CAM per cell (Figure 2.2.A). CAM addition substantially slowed WT growth for about 3 h, after which exponential growth resumed briefly, albeit at a significantly reduced rate compared with before CAM addition (Figure 2.2A). In contrast, after a slight pause following CAM addition, the RM strain continued to grow at the same rate as before antibiotic challenge (Figure 2.2B). Prior to CAM challenge, the growth rate of the RM was about half that of the WT. A slower growth rate, as with the RM strain, can be a consequence of the development of antibiotic resistance capacity in mesophilic bacteria, likely the result of genetic changes associated with this genotype that impair vital cellular functions or confer metabolic burdens (4, 5). While no morphological differences were evident for the RM after CAM addition (Figure 2.2B), CAM challenge had a significant impact upon WT. Substantial morphological

changes were noted in the WT within 30 minutes following the CAM pulse; WT cells appeared to be coccoid-shaped rather than the characteristic rod-shape morphology of exponentially growing *T. maritima*. This observation was consistent with previous reports that mesophilic bacteria form filaments and oval-centered morphologies as a response to beta-lactam antibiotics (41, 51, 98, 105), fluoroquinolones, (27) and CAM (27, 105). The effect of CAM addition on WT morphology was still evident 4.5 h after challenge (Figure 2.2A).

In addition to the CAM challenge in batch culture, chemostat (continuous) culture (80°C, dilution rate of 0.42 h^{-1}) was used to examine the effect of continuous exposure to CAM. This dilution rate corresponded to the approximate growth rate of the WT when it resumed growth following CAM challenge (Figure 2.2A). After establishing mechanical steady states for five or more reactor volumes, CAM was added to the chemostat and feed stream, such that 100 μg/ml antibiotic concentrations (1 pg CAM per cell) could be continuously maintained. As expected, based on MICs, this level of CAM challenge led to washout of the WT strain, while having no noticeable effect on the RM culture (Figure 2.3).

Mutations identified in the 23S rRNA sequence of the RM strain. Because CAM is known to be a translational inhibitor, mutations of 23S rRNA sequence that might be responsible for the relative insensitivity of the RM to CAM were explored. Based upon the genomic sequence of the *T. maritima* 23S rRNA (84), the corresponding sequence of the RM strain was found to contain five mutations (Figure 2.4). The corresponding nucleotide positions/mutations in *E. coli* are used here for reference. The first mutation in *T. maritima* 23S rRNA, located in domain VI, was a G insertion at

nucleotide 2980 (2980_2981*ins*G); this corresponds to nucleotide 2862 in *E. coli* (Eco) 23S rRNA (Eco 2862_2983*ins*G). A second mutation, G907U (Eco G830U), was located on domain II in a site between the GTPase center and the macrolides binding site (7). The other three mutations were all located in domain V, two of which were associated with the peptidyltransferase center loop (PTC loop) (75) G2176A and G2568A (Eco G2057A and G2447A, respectively). Mutations of nucleotide G2176 have been found to be associated with resistance of *E. coli* to both CAM and erythromycin (33), and with resistance of *Mycoplasma* and cutaneous propionibacteria to erythromycin (38, 100). The mutation corresponding to G2568A was initially discovered in the mitochondrial rRNA-CAM resistant strains from *Saccharomyces cereviceae* (31). When engineered into *E. coli*, the corresponding mutation, Eco G2447A, was associated with resistance to CAM at a concentration of at least 81 μg/ml, based upon *in vitro* protein synthesis assays (110). Though the *E. coli* mutation did not show a reduction of *in vivo* translation activity, in reconstituted ribosomes from *Thermus aquaticus*, the corresponding variant was associated with a reduction in the rate of peptidyltransferase activity ranging from 30% to 73% (depending on the assay employed) (92). The third mutation in domain V was at position G2288 (G2288A). This variant, located at the F (final) site of tRNA transit through the ribosome, does not have an *E. coli* counterpart, as the normal base for the corresponding position in *E. coli* (Eco 2169) 23S rRNA is A, and appears to represent a novel mutation. Previous studies suggest that the G2288A variant might affect the rate of final release of the non-aminoacylated tRNA to the cytoplasm (66). This may be a compensatory mutation (4, 76), tuning ribosome function during translation to minimize

the loss of peptidyltransferase activity arising from the double mutation (G2176A and G2568A) at the PTC center (15, 90, 116).

Transcriptional response of WT and RM strains to CAM challenge. Genome-wide differential gene expression was examined for samples of WT and RM obtained before and during CAM challenge from both batch and continuous cultures. Note that CAM challenge in batch and continuous culture represented different physiological contexts for following antibiotic response. In batch cultures, both WT and RM were allowed to enter exponential phase, growing at the maximum rate attainable for each strain on SSM at 80°C, prior to CAM addition (when cell density reached ~10^7 cells/ml). For the continuous culture, the dilution rate was set at 0.42 h^{-1}, compared to an estimated washout rate of approximately 1 h^{-1} for the WT strain on this medium (unpublished data). CAM (100 μg/ml) was added to a steady state continuous culture at 10-fold higher cell density than in batch culture so that the amount of CAM/cell in the chemostat was 10-fold lower. The use of a steady state approach allows the direct comparison of WT and RM strains at the same growth rate, also eliminating any potential confounding factor that might be associated with cell density dependent effects or by the accumulation of other metabolites during batch cultures. The dilution rate used, 0.42 h^{-1}, corresponding to a doubling time of 100 min, mimicked the doubling time observed during recovery of the WT for CAM challenge in batch culture (see Figure 2.2A). However, this dilution rate lead to washout of the WT in the chemostat, suggesting that the WT recovering from CAM challenge in batch culture may represent a resistant mutant (see above).

Differences between RM and WT transcriptomes prior to CAM challenge. The contrast between the transcriptional profiles of RM and WT strains prior to CAM exposure was significant. In continuous culture, only 19 ORFs were differentially expressed (7 up/12 down) 2-fold or more in the RM strain compared to WT (Table 2.1, Table 2.2 and Figures 2.5 and 2.6), with most of the up-regulated ORFs belonging to an operon (TM0979-TM0983) of unknown function. However, in batch culture, where growth rates could be maximized for the given medium and growth conditions, 131 ORFs (75 up and 56 down, in the RM compared to the WT) were differentially expressed 2-fold or more between the strains. One of the sharpest differences between RM and WT from the perspective of their transcriptomes was the significant up-regulation of ribosomal protein genes in the RM strains (see Figure 2.7). Among them were the genes for 32 ribosomal structural proteins, representing about 2/3 of the identifiable ribosomal proteins encoded in the *T. maritima* genome (84). Other translation-associated genes up-regulated in the RM strain include RNAse P (TM1463), IF-1 (TM1477), IF-3 (TM1590), EF-Tu (TM 1501), EF-G (TM1502), methionine aminopeptidase (TM1478) and SecY (TM1480). Genes involved in polyamine synthesis (TM0654) were up-regulated in RM, suggesting a pre-conditioning of this strain to CAM challenge which could be important in offsetting the deleterious effect of CAM on RNA stability or in reducing oxidative stress (see below). Further analysis revealed the up-regulation of heat shock operons, including DnaJ-GrpE-Hrc (TM0849-51) and GroES-GroEL (TM0505-0506), in the WT strain, while two cold shock genes (TM1683, TM1874) were up-regulated in RM. As a consequence of the mutations in 23S rRNA (and, perhaps, others elsewhere in the genome), differences between the unchallenged RM and WT strains indicated that the

RM strain had developed a "CAM-ready" state that warded off the normally deleterious impact of antibiotic exposure, perhaps arising from compensatory modifications that minimized any adverse consequences of primary mutations. Compensatory evolution is usually more common than reversion, since the mutation rate for the former is higher (because of the larger mutational target) than for the latter (4). Since the RM was selected by extensive serial passages in CAM, enrichment for compensating mutants over less resistant strains or revertants would have resulted. These differences presumably reflect, at least in part, the impact of the five 23S rRNA mutations found in the RM strain, though additional contributions from as yet unidentified mutations of the *T. maritima* genome cannot be ruled out.

Differences between RM and WT transcriptomes following CAM challenge. Figures 2.5 and 2.6 summarize genome-wide differential transcription occurring within and between the two strains grown in batch and chemostat culture for comparisons at 0, 5, and 30 minutes after CAM challenge. For details, see Table 2.1. The response to CAM in the WT was immediate, with 274 and 256 ORFs up-regulated in batch and continuous culture, respectively, within 5 min after challenge. The RM strain, in contrast, was initially insensitive to the CAM challenge, perhaps reflecting a pre-conditioned state arising from mutations in 23S rRNA and other genomic loci. Among the 8 genes that were differentially expressed in the RM upon CAM challenge, a small putative peptide (TM1316) was down-regulated 2-fold. In the RM at 5 min post-challenge, 60 ORFs (32 up/28 down) were differentially expressed, compared to 274 ORFs (139 up/ 135 down). Also, the response of the RM at 30 min was comparable to that observed in the WT at 5 min.

There were many distinct features between the WT and RM response to CAM in batch culture (see Figure 2.5). As an overview, all genes differentially expressed at 5 min and 30 min post-CAM challenge were categorized according to the COG database (108) (see Figure 2.8). It is important to point out that many differentially expressed transcripts, especially after 30 min post-challenge, could not be assigned to any COG category. In the case of the WT, the initial response at 5 min was characterized by changes in gene expression primarily related to amino acid transport and metabolism (E), translation (J), and carbohydrate transport and metabolism (C). On the other hand, the RM strain, in addition to differential transcription of genes in the same categories as the WT, genes associated with replication, recombination, and repair (L) also responded. It is interesting that several down-regulated ORFs in the locus TM1806-TM1811 (e.g., TM1807, TM1808 and TM1810) contain CRISP-associated sequences (30 bp repeat palindromic elements interspaced with a variable and non-repetitive 39-40 bp spacer sequence) that have been implicated in DNA mobilization (80). Note that an *E. coli* streptomycin-inducible mutator strain, dependent on RuvABC, has been described (9); perhaps, the RM strain here contained a CAM-inducible mutation that required the response of this same DNA repair/recombination pathway.

Figure 2.6 provides an alternative perspective (using Venn diagrams) of overall differential transcription in the WT and RM before and during CAM challenge. One interesting finding was that the transcriptional profiles of the WT and RM were more different after 5 minutes post-CAM challenge (430 ORFs), than at 30 min (248 ORFs), suggesting the RM response was a up to some extend a delayed version of the WT response.

CAM-induced polyamine synthesis and oxidative stress response in *T. maritima*: Differential transcription of ORFs associated with a spermidine synthase operon was observed (TM0654-TM0656) (Figure 2.9). In bacteria, polyamines are associated with a variety of functions, such as modulation of DNA, RNA and protein synthesis (55). They are also necessary for normal cell growth, and have been implicated as essential factors in osmoregulation, response to pH, oxidative stress, control of rate of growth and induction of death in late stationary phase (6, 26, 61, 112, 124). In hyperthermophilic microorganisms, polyamines have been associated with the stabilization of nucleic acids at high temperatures (109) as well as being an essential component of *in vitro* protein synthesis systems (73, 114). In *T. maritima MSB8,* the presence of polyamines of diverse chain lengths has been described, and a direct correlation between the presence of polyamines and cellular response to high temperatures has been shown (126).

Upon exposure of certain bacteria to bacteriocins and antibiotics, including CAM, (2, 32), production of the anion superoxide (O_2^-) has been detected. In *E. coli,* it has been demonstrated that exposure to oxidative stress, induced by hydrogen peroxide exposure, is linked to accumulation of intracellular levels of polyamines. This accumulation seems to be associated with the activation of the polyamine synthetic pathway. Accumulation of polyamines has been directly linked to the production of proteins, such as AphC, KatE, KatG and OxyR, which are known to participate in the oxidative response of *E. coli* to Reactive Oxygen Species (ROS). This induction can be directly associated with increased survival to oxidative stress(62, 112). As seen on Figure 2.9, TM0654 (Spermidine

synthase, EC:2.5.1.16) and TM0655 (S-adenosylmethionine decarboxylase, EC:4.1.1.50) were up-regulated in the WT upon exposure to CAM. This response was apparently independent of growth rate, growth condition and relative dosage of CAM. Related genes in the polyamine synthetic pathway, such as TM1658 (S-adenosyl-methionine synthase) and TM1873 (Ornithine decarboxylase), were not differentially transcribed for the conditions tested. Although it is known that these genes are highly regulated, it is also true that they are associated with synthesis of metabolites involved in many essential biochemical processes, including synthesis of polyamines, unsaturated fatty acids, siderophores, modified tRNA bases, N-acylhomoserine lactones and other signaling molecules (36). Therefore, one would expect to find complex regulatory networks involving dedicated two component signaling systems and anti-zymes that regulate these genes (70, 71).

Divergently transcribed from the spermidine synthase genes (Figure 2.9) is a putative operon, comprised of TM0657 (rubryrethrin), TM0659 (rubredoxin) and TM0658 (neelaredoxin). TM0657 is co-located with the *P. furiosus* rubryrethrin in a mix-branched phylogenetic tree of bacterial/archaeal rubrerythrins. The functional role of the *P. furiosus* homologs to the aforementioned genes has been recently demonstrated *in vivo* in *E. coli*. (42). In addition to these genes, NAD(P)H rubredoxin oxidoreductase (NROR) is required to complete the detoxification pathway for ROS in anaerobic microbes (42, 119). As shown on Figure 2.9, these genes (TM0657-TM0659) are up-regulated upon exposure to CAM, especially in the continuous culture. This set of genes is among the most highly transcribed in *P. furiosus* such that differential transcription is difficult to assess using cDNA microarrays (119). These genes were also among the most abundant

transcripts in *T. maritima*, comparable to the signal intensities observed for some ribosomal proteins.

The up-regulation of TM0472 (glutamine amidotransferase subunit pdxT) and TM0473 (pyridoxal biosynthesis lyase) (see Figure 2.10) further indicated oxidative stress; these two genes are associated with the synthesis of pyridoxin. A similar response was detected in *Schizosaccharomyces pombe* upon exposure to hydrogen peroxide (22). This suggests an increased requirement for B vitamin-derived coenzymes and pyridoxal phosphate. *In vitro*, pyridoxin can quench singlet oxygen and protect against superoxide-mediated damage. (14, 87). The antimicrobial effect of CAM is not always associated with direct binding to the PTC (13, 86). The binding site with highest affinity to CAM in archaea (*Haloarcula marismortui*) is known to require a higher concentration of CAM and is located at the entrance of the nascent peptide exit tunnel (44, 106). Therefore, the detection of antimicrobial activity of CAM in archaea was not expected. However, several archaeal strains, including *Methanococcus voltae, Methanococcus. vannielii, Methanococcus deltae* and *Methanobrevibacter smithii*, are sensitive to CAM because of the presence of the aryl nitro group in this antibiotic, a moiety that under anaerobic conditions acts as an oxidizing agent (13, 86). Given the high CAM levels used here, this effect cannot be ruled out for *T. maritima*. It is interesting that this response (Figure 2.9) was not noted in the RM, with the exception that TM0654 (SpeE, spermidine synthase) and TM0658 (Rubredoxin) (see Figure 2.11) were up-regulated in continuous culture CAM challenge.

Chloramphenicol and induction of flagellar genes. It has been reported that there is an inverse relationship between the expression of flagella and stress, including osmotic stress, exposure to antimicrobials or cationic antimicrobial peptides (8, 68). On the other hand, it has been reported that under carbon starvation there is induction of flagella biosynthesis (72) and also examples have been described in which polyamine-directed systems are associated with up-regulation of flagellar genes (99). In *Proteus mirabilis*, putrescine acts as an extracellular signal required for the transition to a swarming phenotype (107). This swarming phenotype is associated with, among other things, an increase in cell size and number of flagella (99). In *T. maritima* many flagellar genes were differentially expressed for WT and RM at certain points (see Figures 2.12 and 2.13). Among the genes most highly up-regulated during CAM exposure was flagellin FlgL (TM0758), the main structural "monomer" of flagella, which was up-regulated as much as 7.5-fold after 5 min post CAM challenge in the WT. The up-regulated flagellar genes belonged to the assemblage pathway: the F and P ring, motor switch proteins, basal body, filament and flagellar hook molecules. It is interesting that among the other groups of genes being down-regulated in the WT after CAM challenge (see Figure 2.13) was a homolog (TM0132) of flagellin, indicating the capability of *T. maritima* to combine different flagellin monomers or produce flagella with an alternative building block. Another point of interest was the moderate up-regulation of FlgM, an anti-sigma 28 factor, that allows the orderly transcription and assembly of the class I (Motor Switch, Basal Body) and class II (Motor Switch-Proximal Rod-Basal Body) flagellar genes before the transcription of the genes of class III (Hook and filaments) (3). Taken together, there was an apparent uncoupling of the flagellar assembly process in *T.*

maritima, based on reports for other bacteria; genes from class III and class II were co-expressed, while the only genes that are being down-regulated were from class II. This might indicate an alternative regulatory strategy or an impact of CAM challenge. Finally, *T. maritima* has a different genomic organization of flagellar genes compared to mesophilic bacteria, for instance *E. coli* class I flagellar are not present in the *T. maritima* genome.

Response of regulatory proteins and CAM challenge. There were distinctive features in the response of global regulatory proteins, such as sigma factors, in the RM compared to the WT. RpoD (σ^A) and RpoE (σ^E) in the WT were responsive at 5 minutes to CAM (see Figure 2.14). The physiological implications of this rapid response can be corroborated by the concomitant differential transcription of 274 genes in the WT. Only 60 genes were differentially transcribed in the RM. In batch culture, RpoD and RpoE were up-regulated in the RM after 30 min following CAM challenge. In Gram-negative bacteria, reduction in cell permeability has been linked to antibiotic resistance through modification or reduction of expression of an outer membrane porin (20, 21); this may be the case here for the RM. The other possibility is that the mutations observed in the 23SrRNA hampered the binding of CAM to the ribosomes, which is frequently observed in antibiotic-resistant bacterial mutants (see Figure 2.4) (75).

RpoE and RpoD were previously observed to be differentially transcribed in *T. maritima* during heat shock, stationary phase and biofilm formation (57, 93, 94). These seem to be part of a universal stress response feature in *T. maritima*. In only the WT was there down-regulation of the putative anti-sigma factors TM1442, TM1081, and TM0733

upon exposure to CAM, which could amplify the antibiotic response (53). Even after 30 min exposure to CAM, there was no significant change in the expression of these genes in the RM.

Bacteria possess proteins with superficial similarity to eukaryotic histones; these proteins have low molecular weight and an electrostatic charge that underlie their DNA binding ability. It has been shown that such proteins contribute to the control of gene expression in bacteria (29). Upon exposure to environmental stresses, changes in the level of the bacterial DNA supercoiling, mediated by gyrases, topoisomerases and histone-like proteins, have been reported (23, 120). In some conditions, this process is essential for the induction of supercoiling-dependent genes (23). Based on the current annotation of *T. maritima* and BLAST searches, the only identifiable histone-like protein in *T. maritima* an HU protein (TM0266). As seen in Figure 2.14, CAM challenge induced the up-regulation of the HU DNA binding protein only in the WT. However, this appears to be an isolated event because there was no differential transcription of any topoisomerase, helicase, or reverse gyrase. However, significant down-regulation of GyrB, the ATPase subunit of the gyrase was observed, although no change in expression levels was noted for GyrA.

In Figure 2.15, the transcriptional profiles off all the aforementioned genes are shown for the continuous culture. As expected, none of these genes in the RM were differentially expressed, although TM0908 (sigma factor 28), associated with the synthesis of flagella in *E. coli* (3), and TM1451 (sigma A) was up-regulated in the WT continuous culture after CAM challenge.

Response of "heat shock" and "cold shock" proteins to CAM challenge: More than a decade ago, it was recognized that translational inhibitors can be classified based upon whether the induction of "cold shock proteins" or heat shock proteins are induced (115). Since then, the association of CAM with the induction of cold shock proteins has been demonstrated for *Haemophilus influenzae* by using proteomics (34), and by microarray analysis for *Mycobacterium tuberculosis* (18) and *Streptococcus pneumoneae* (85). As shown in Figures 2.16 and 2.17, this also seems to be the case in *T. maritima*, where the cold shock protein genes CspC (TM1874 and TM1863) were both up-regulated. In the case of the RM, as expected, no changes were detected in these genes for continuous culture conditions. The general role of cold shock proteins is as RNA chaperones, preventing the formation of inhibitory secondary structures at sub-optimal lower temperatures (91). On the other hand, induction of heat shock proteins have been reported in antibiotics, such as kanamycin, that induce mis-translation, and it is known that CAM induces these effects in *E. coli* (111). In *Bacillus subtilis*, using sub-lethal doses of different translational inhibitors, heat shock proteins were induced for up to 60 min after exposure (69). However, in the case of WT *T. maritima*, the induction of these genes seems to be part of only the initial response to CAM. In the case of the RM strain, the induction of the heat shock protein chaperones, DnaK, GroEL and GroES, is maintained to beyond 30 minutes after exposure to CAM. The induction of heat shock genes is part of an unspecific or secondary response to this antibiotic in bacteria (111).

Figures 2.16 and 2.17 also show the transcriptional profile of TM0504, which corresponds to the signaling molecule triggering exopolysaccharide production in *T.*

maritima (58). TM0504 is encoded in the complementary strand of tmRNA, which is a highly structured RNA molecule associated with the process of ribosomal rescue and degradation of partially translated nascent peptides (118, 121). Up-regulation of tmRNA has been reported in *B. subtilis* upon exposure to environmental stresses (82). The role of tmRNA in the response to antibiotics has been demonstrated by the creation of knock-out mutants hypersensitive to antibiotics (118). Little is known about the transcriptional regulation of tmRNA. In *E*. coli, specific endonucleases have been linked to the cleavage and degradation of this molecule (25). In *Caulobacter crescentus*, tmRNA is linked to the proper regulation of the cell cycle, and the levels of this transcript are tightly regulated in a process that is intrinsically dependent on the RNAse R activity and the levels of SmpB protein (50). Here, the up-regulation of SmpB was seen upon exposure to CAM in the RM in batch culture. In the case of the WT, it seems that there is initially down-regulation of SmpB, followed by up-regulation over following 30 min as the culture regroups or recovers from the CAM challenge.

Response of ribosomal proteins, synthesis of nucleotides and ATP synthase. The association between accumulation of mRNAs from ribosomal protein operons and challenge with translational inhibitors is observed across all bacterial groups tested, including *E. coli, S. pneumoneae, H. influenzae* and *B. subtilis* (18, 34, 35, 69, 85, 101). This response is usually accompanied with the down-regulation in synthesis of amino acid and up-regulation of transcriptional factors (e.g., IF-3, greA, Ef-Tu, NusA). In the case of the WT in batch culture, the effect was quite clear. The response to CAM also included accumulation of mRNAs for ribosomal protein operons and the down-regulation

of genes associated with the biosynthesis of amino acids (in particular, Met, His, Val, Leu, Ile, Gly, Lys, Thr, Ser, Glu, Try, Cys). (See supplementary Tables). This response was also accompanied by the down-regulation of the F1/F0 ATP synthase structural components (see Figure 2.18), and down-regulation of the cellobiose uptake transporter (TM1219-TM1226); cellobiose was the primary carbon and energy source used here.

The transcriptional profiles of genes associated with the salvage and *de novo* synthesis pathway of purines in *T. maritima* for WT and RM are shown in Figure 2.19. Down-regulation of these purine and pyrimidine synthesis genes in response to translational inhibitor was noted in *B. subtilis* (35). The response was apparently independent of a stringent response as judged by the absence of the negative regulation of components of the translational apparatus including rRNAs, tRNAs, ribosomal proteins, and translation factors (69).

Analysis of unique responses in the mutant strain. In addition to the response delay observed in the mutant strain to CAM challenge, there were interesting features indicative of additional mechanisms of resistance besides the reported mutations at the 23S rRNA. As an example, TM0119 was more highly expressed in the mutant strain and was induced in both strains upon exposure to CAM (see Figure 2.20). TM0119 belongs to the family FmdA_AmdA of formamidases and acetamidases, and it has 33%/48% identity/similarity to an L-amidase with extremely broad specificity isolated from *Ochrobactrum anthropi* NCIMB 4032, which has been used for the synthesis of complex peptides (60). It has been reported that chloramphenicol hydrolases provide a mechanism of resistance in the natural CAM-producing strain *Streptomyces venezuelae* (81).

Unfortunately, no amino acid sequence data are available for this enzyme. It is not clear whether the unusual transcriptional profile for TM0119 is a consequence of an unknown metabolic pathway induced by CAM challenge. TM0118 is a putative ribonucleotide reductase with conserved RNR_1 domain (78), implicated in the reductive synthesis of deoxyribonucleotides from the corresponding ribonucleotides. TM0120, which followed transcription of patterns of TM0119, corresponds to a putative nitroreductase that employs FMN (Flavine mononucleotide) as a cofactor in the reduction of nitrogen containing compounds. If TM0119 is proven to be active with aliphatic amides, it might have biotechnological potential in the bioremediation of acrylamide, and acrylate contaminated soils (83).

Conclusions. The contrast between the two strains prior to CAM challenge could be related to inefficiencies resulting from the point mutations in the 23S rRNA to minimize the impact of CAM and the possible strategy of overproducing ribosomal proteins to compensate. This disparity in results between continuous and batch cultures may be a consequence of the significant difference in growth rates between the two strains in batch culture prior to the CAM challenge, with doubling times of 37 min and 64 min for the WT and RM strains, respectively (Figures 2.2.A and 2.2.B). Transcriptional profiles of the WT strain in batch and co-culture upon CAM challenge showed a marked down-regulation of genes associated with the synthesis of macromolecules, amino acid and nucleotide biosynthetic genes. Stress response-like behavior was observed, given the up-regulation of genes encoding cold shock proteins, chaperones, ribosomal proteins sigma factors, polyamine synthesis pathways and oxidative response elements. In the

RM, stress response was delayed or minimized. Given the high level of resistance observed in the RM additional mutations in the *T. maritima* RM cannot be ruled out.

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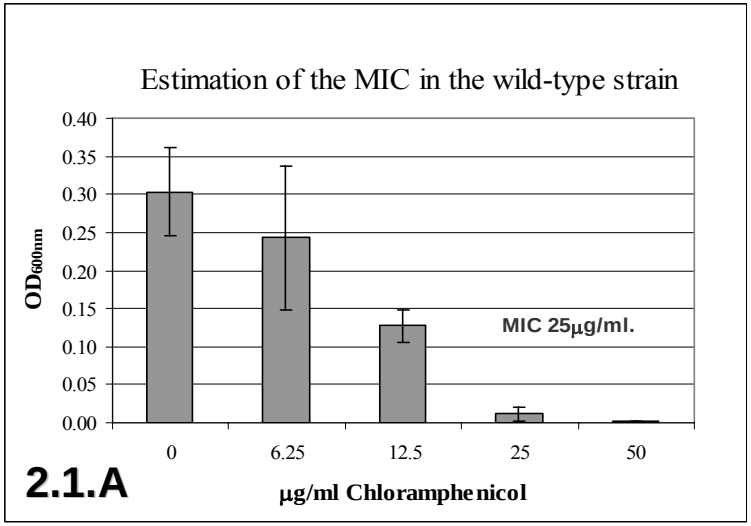


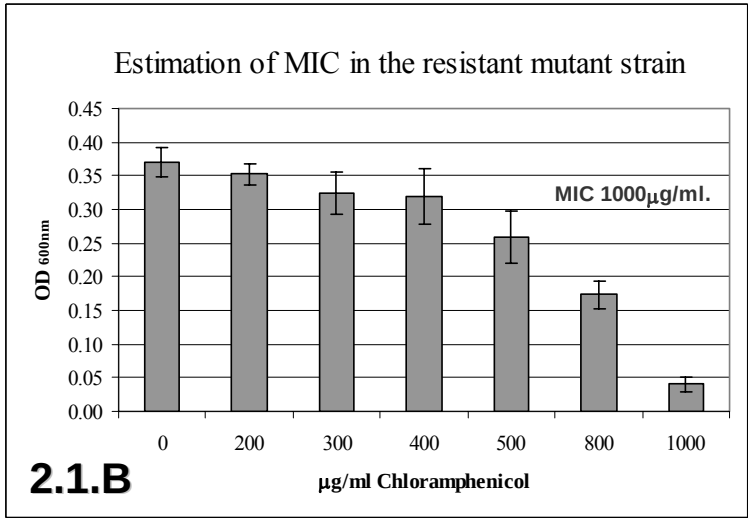


Figure 2.1: Estimation of the minimum inhibitory concentration (MIC) of CAM for the WT and RM strain of *T. maritima.* Cultures were grown in SSM at 80°C for 24 hours with increasing concentrations of CAM. MIC was defined as the concentration of CAM in which the absorbance at 600nm was less than 0.05.

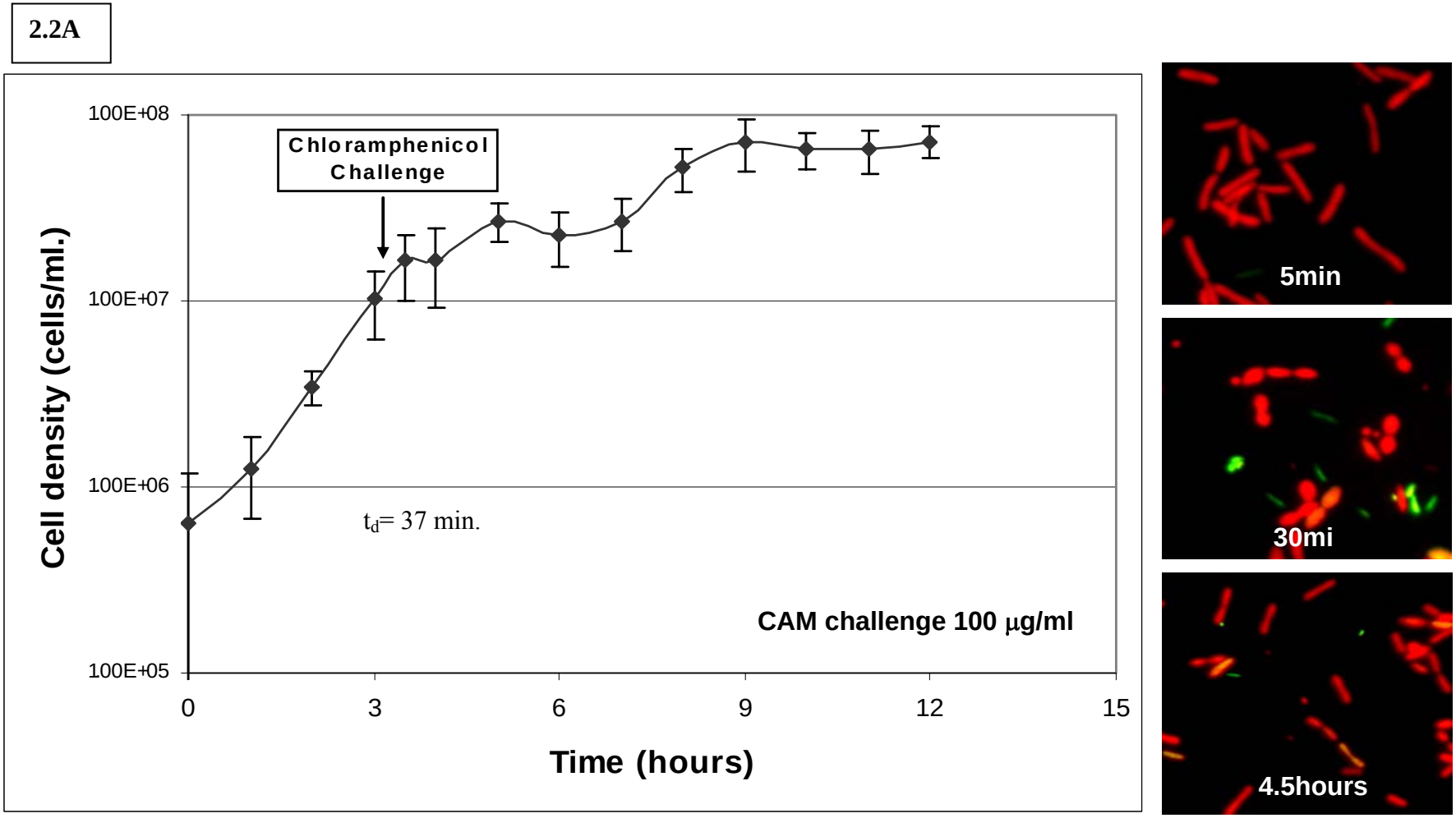


FIGURE 2.2: Chloramphenicol challenge of wild-type (WT) *T. maritima* (A) and resistant mutant (RM) (B). The mutant strain grew more slowly than the wild-type prior to CAM challenge but showed no morphological or growth rate changes after challenge compared to the response of wild-type culture; wild-type morphology returned to pre-challenge state within several hours.

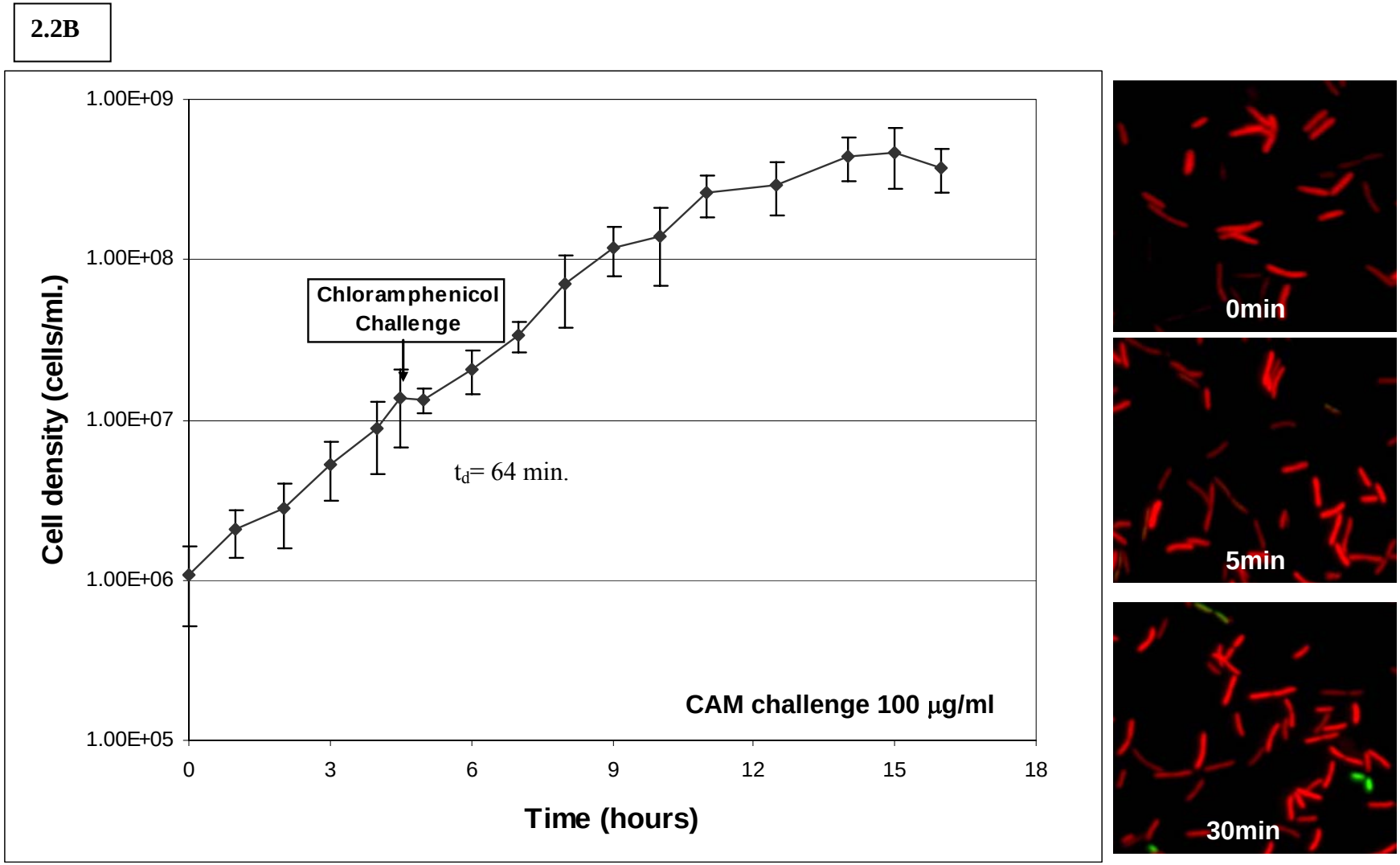
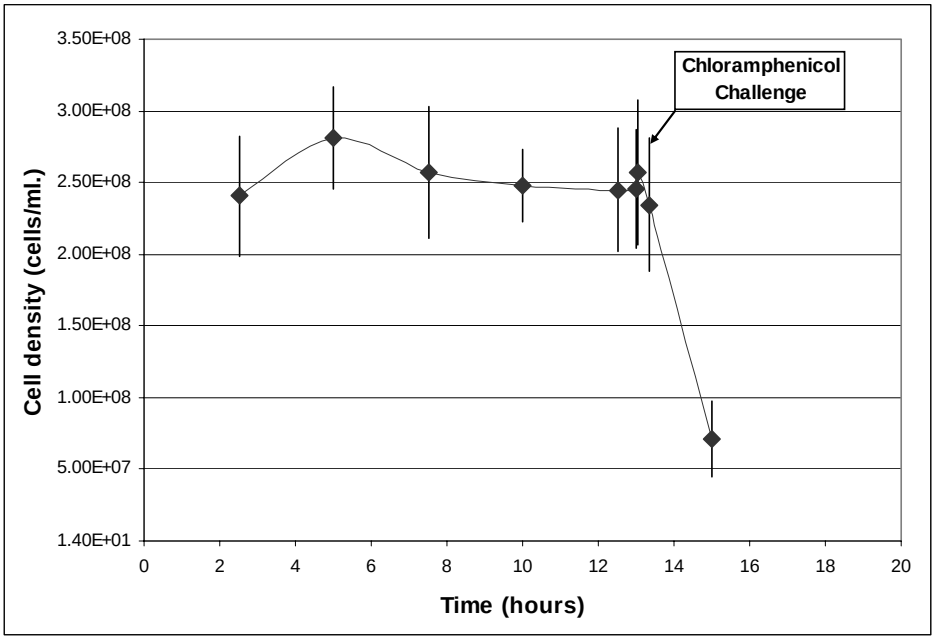


FIGURE 2.2: Chloramphenicol challenge of wild-type (WT) *T. maritima* (A) and resistant mutant (RM) (B). The mutant strain grew more slowly than the wild-type prior to CAM challenge but showed no morphological changes after challenge compared to the response of wild-type culture; wild-type morphology returned to pre-challenge state within several hours.

A



B

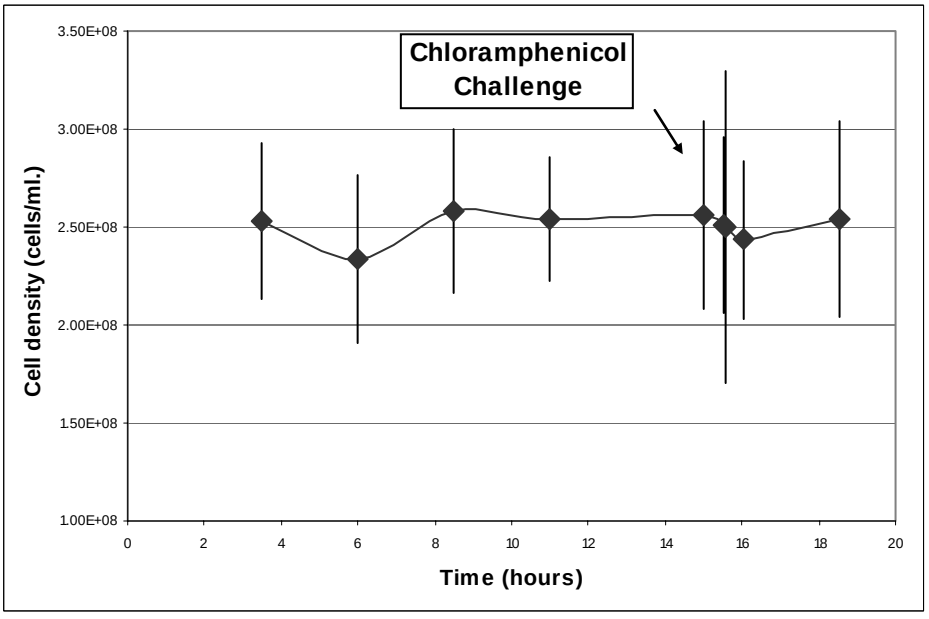
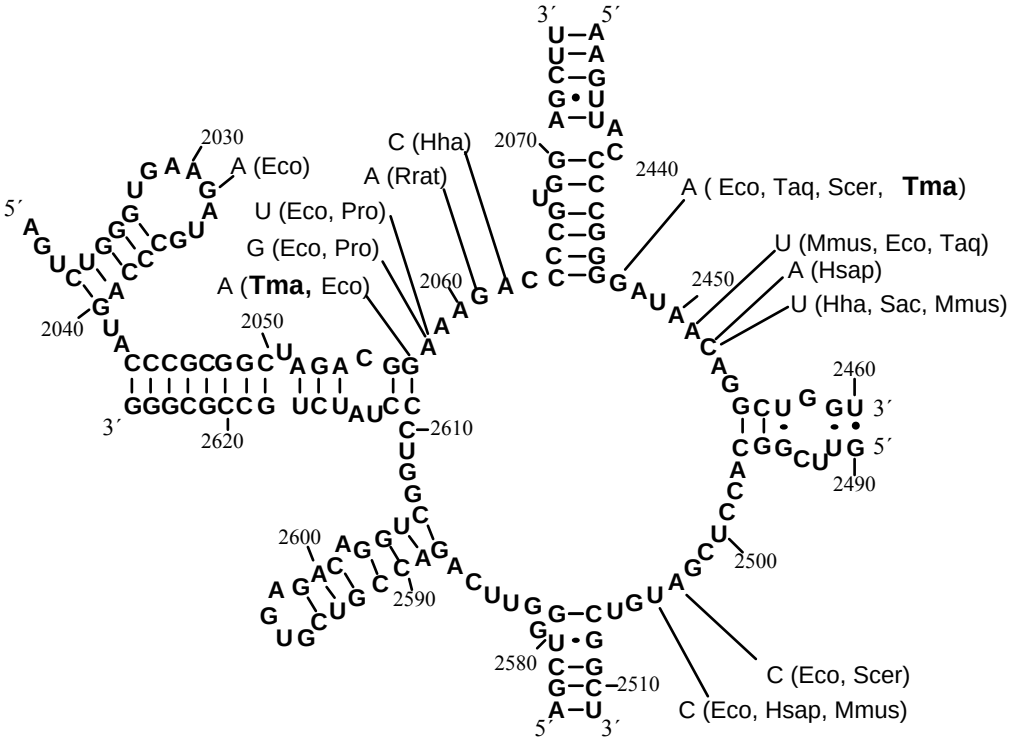


Figure 2.3: Response of *T. maritima* (A) and *T.maritma* RM strain (B) to a 100µg/ml chloramphenicol challenge in continuous culture conditions at a dilution rate of 0.42h^{-1}.

Figure 2.4: Point mutations identified in the nucleotide sequence of 23S rRNA of chloramphenicol-resistant mutant of *Thermotoga maritima* (Tma). Mutation sites are referenced to *E. coli* (Eco). Note that G2288A in *T. maritima* corresponds to an adenine at location Eco 2169. Mutations identified in the 23S rRNA PTC loop related to chloramphenicol resistance are shown in the schematic below for Tma and other organisms/organelles: G2032A in Eco (30); G2057A in Eco (33) and Tma (this work); A2058G and A2058U in *Propionibacterium* sp (100) and Eco (30); G2061A in Rrat (67), A2062C in Hha (77); G2447A in Eco (110), Scer (31), Taq (92) Tma (this work); A2451U in Mmus (63), Eco (110), Taq (92); C2542A in Hsap (16); C2542U Hha (77), Sac (1), Mmus (104); A2053C in Scer (31), Eco (117); U2054C (16, 117) and Hsap (63).

Location (Tma)	Location (Eco)	23S rRNA domain	Previously noted in other organism(s)?
2980_2981 *ins*G	2862_2863 *ins*G	VI	No
G2568A	G2447A	V	Yes: Eco (110),Taq (92), Scer (31)
G2288A	A2169	V	No
G2176A	G2057A	V	Yes: Eco (33)
G907U	G830U	II	No



KEY: Microorganisms: Eco (*Escherichia coli*), Taq (*Thermus aquaticus*), Hha (*Halobacterium halobium*), Sac (*Sulfolobus acidocaldarius*), Scer (*Saccharomyces cerevisiae*); Prs *Propionibacterium sp.* Mitochondria: Rrat (*Rattus rattus*), Mmus (*Mus musculus*), Hsap (*Homo sapiens*).

FIGURE 2.5: ORFs differentially expressed in *T. maritima* wild-type (WT) and chloramphenicol-resistant mutant (RM) during antibiotic challenge in batch and continuous culture.

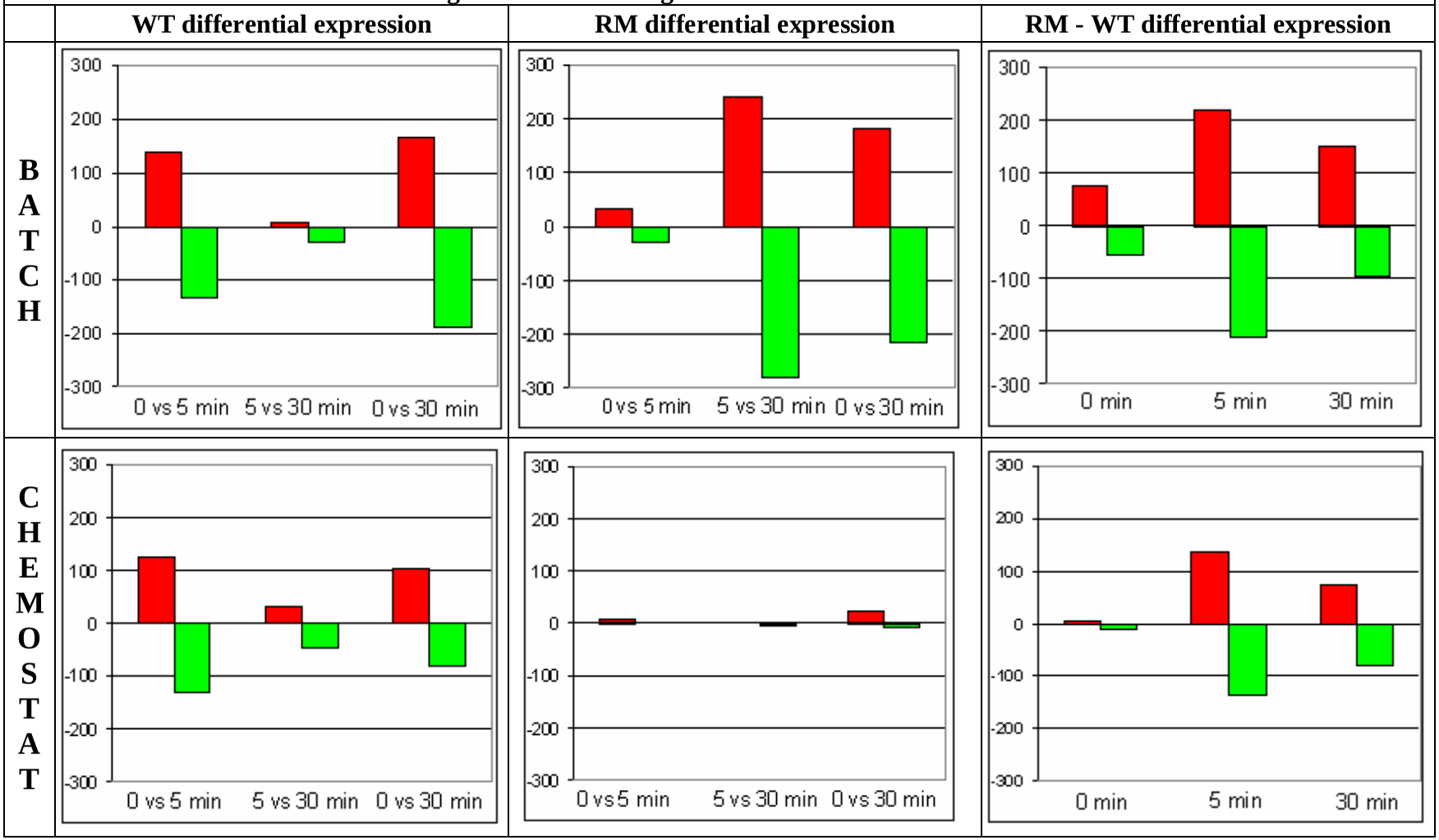
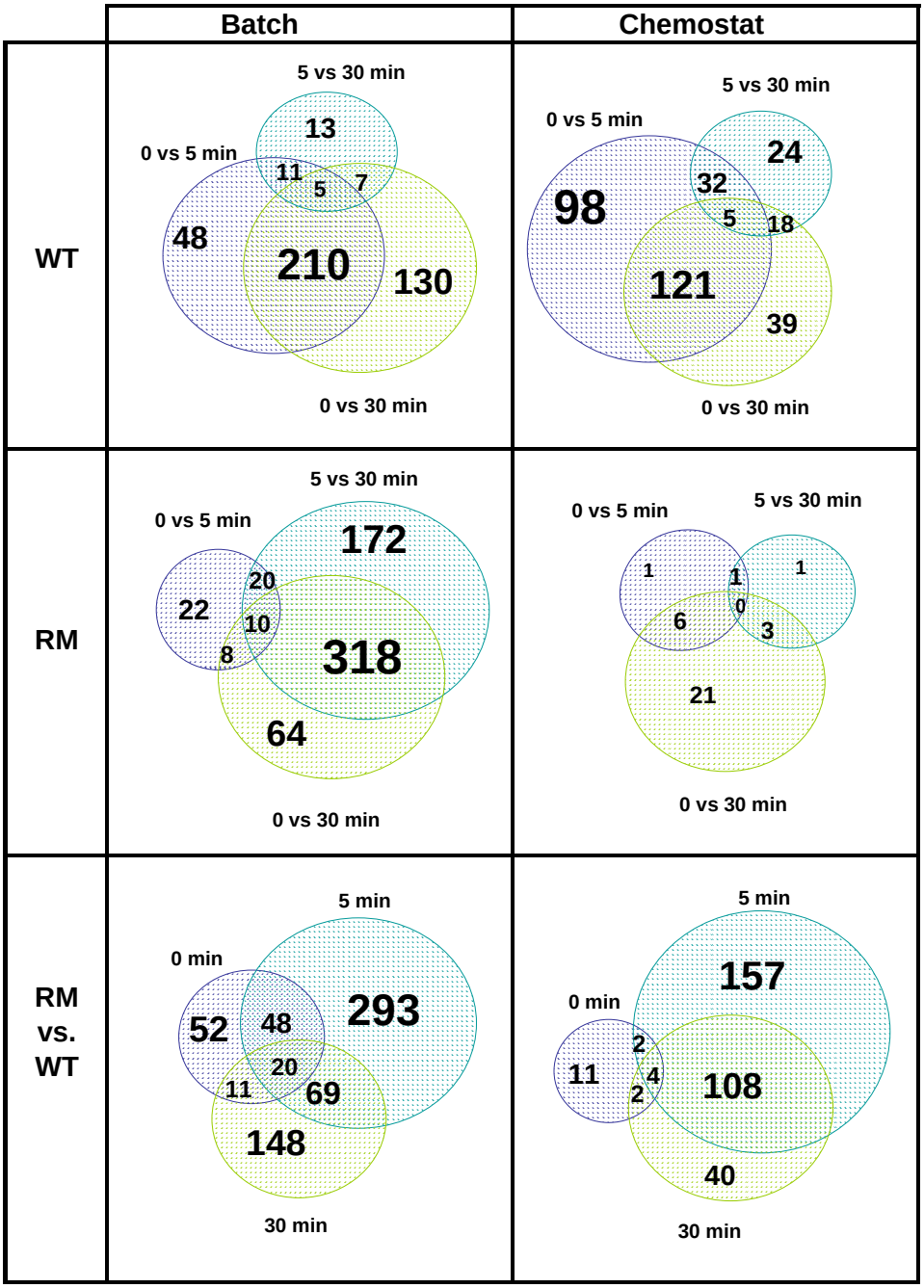


Figure 2.6: Distribution of differentially expressed ORFs for batch and continuous culture comparisons shown in Figure 2.5. For example, for the WT 0-30 min comparison, 130 ORFs were differentially expressed 2-fold or more, while only 5 ORFs were differentially expressed at this level for all three (WT 0-5, 5-30, and 0-30) dynamic contrasts.



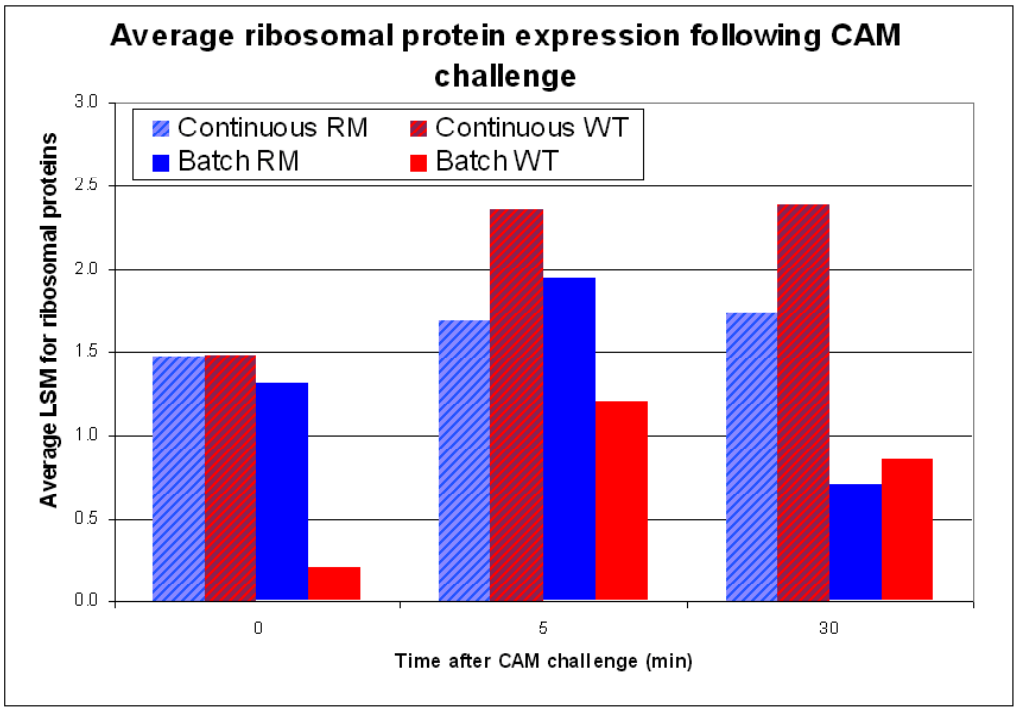


Figure 2.7: Least square means (LSM) of ribosomal protein inventory of *T. maritima* (WT) and CAM resistant mutant (RM) in batch and continuous culture before and during CAM challenge at 100µg/ml. LSM determined through mixed effects model analysis of cDNA microarray differential expression data.

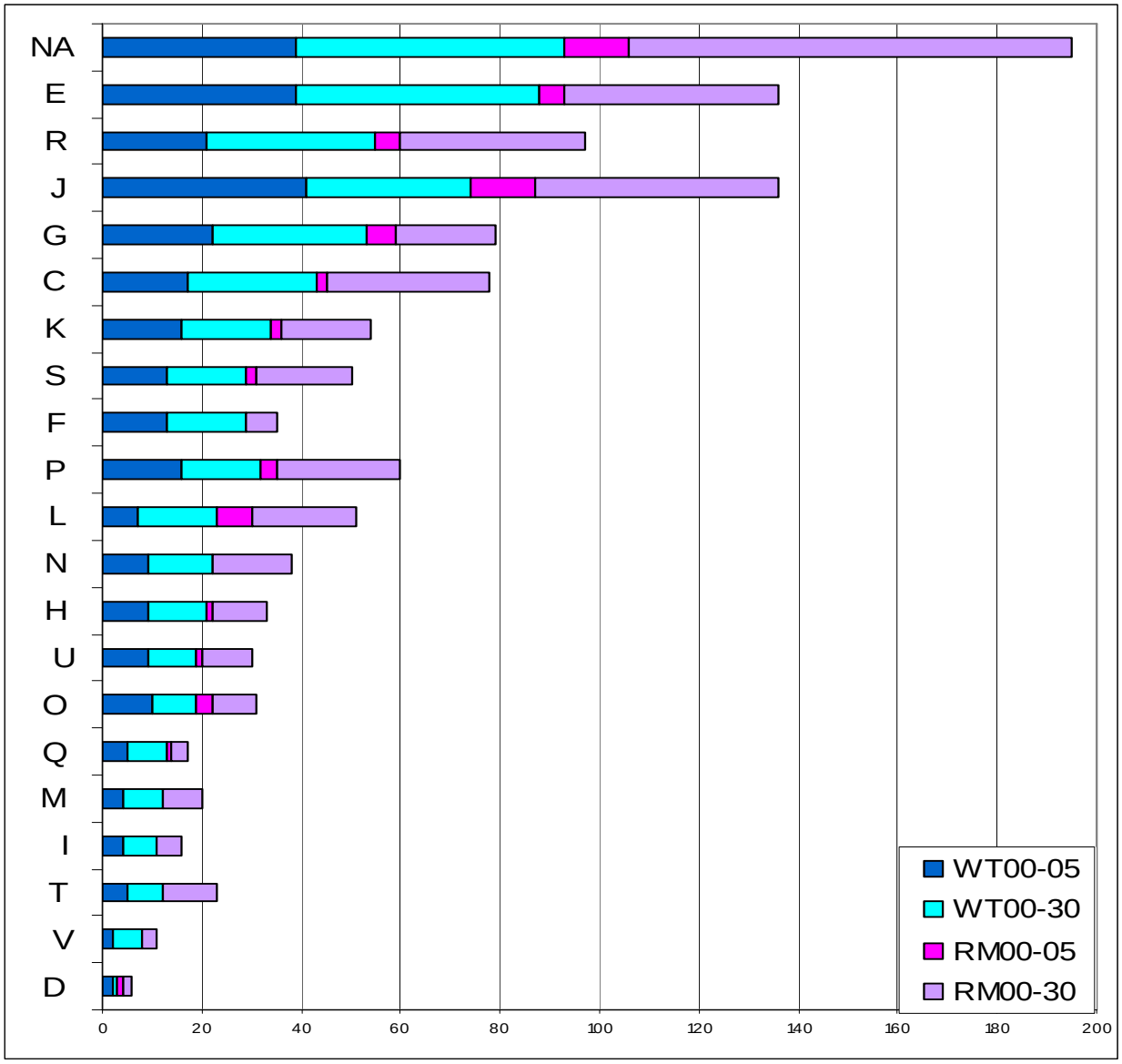


Figure 2.8: COG category distribution of function for ORFs affected by CAM challenge in batch culture condition (108)**.** The number of occurrences is reported based on functional categories for the following comparisons: WT00-WT05, WT00-WT30 and RM00-RM05 and RM00-RM30. Legend for functional categories: NA= Not assigned to any cluster of orthologs groups, E= Amino acid transport and metabolism, R= General function prediction, J= Translation, G= Carbohydrate transport and metabolism, C= Energy production and conversion, K= Transcription, S= Unknown function, F= Nucleotide transport and metabolism, P= Inorganic ion transport and metabolism, L= Replication, recombination and repair, N= Cell motility, H= Coenzyme transport and metabolism, U= Intracellular trafficking and secretion, O= Post-translational modification, protein turnover and chaperones, Q= Secondary metabolites biosynthesis transport and catabolism, M= Cell wall/membrane biogenesis, I= Lipid transport and metabolism, T= Signal transduction mechanisms, V= Defense mechanisms, D= Cell cycle control.

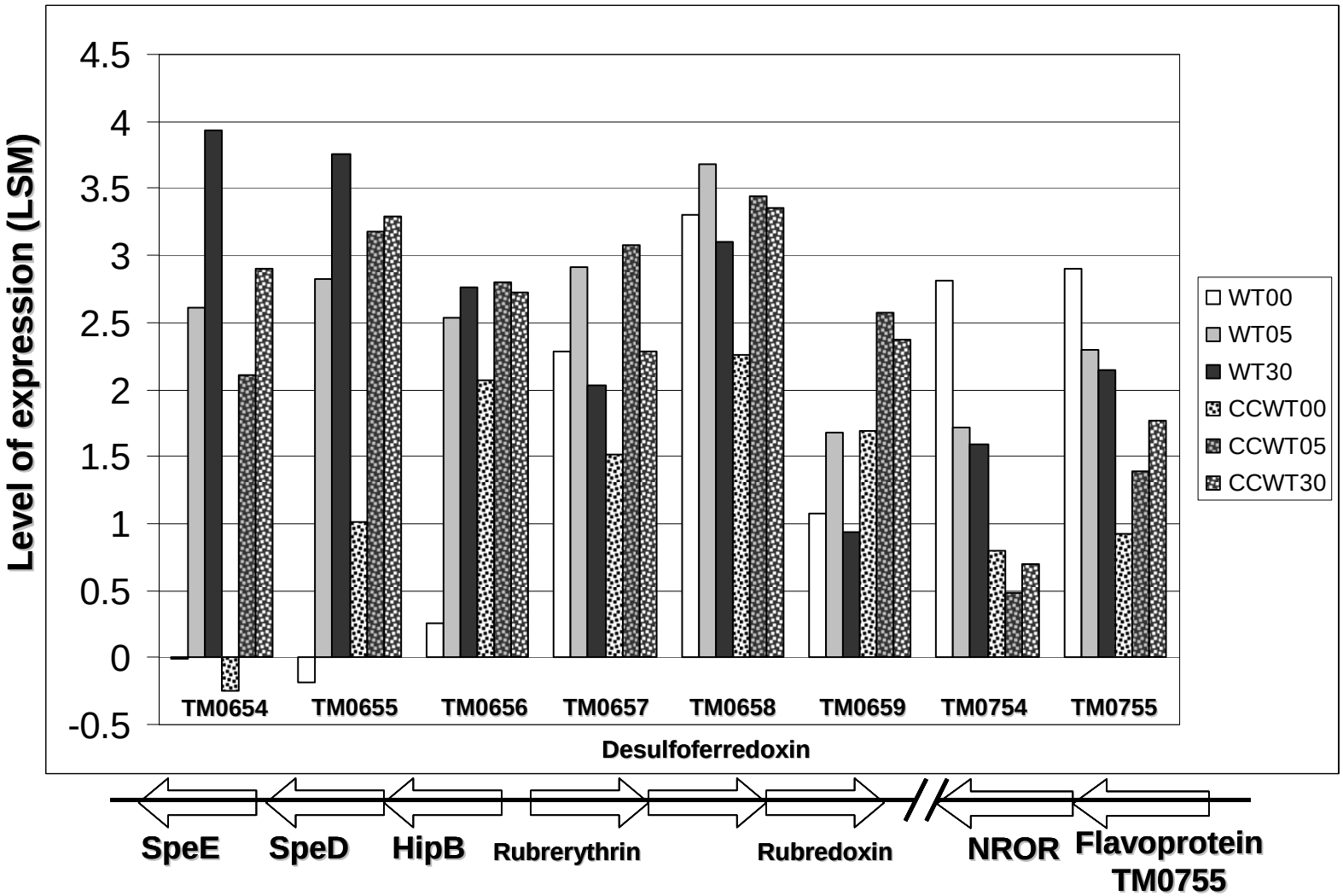


Figure 2.9. Transcriptional profile of genes associated to spermidine synthase in *T. maritima* (WT): The present figure provides the relative levels of expression of the genes located between TM0654 and TM0659 and genes related to the superoxide reductase ROS detoxification pathway previously described in *P. furiosus* (42). That seems to be responsive in continuous culture, and to a lesser extent in batch culture to the CAM challenge.

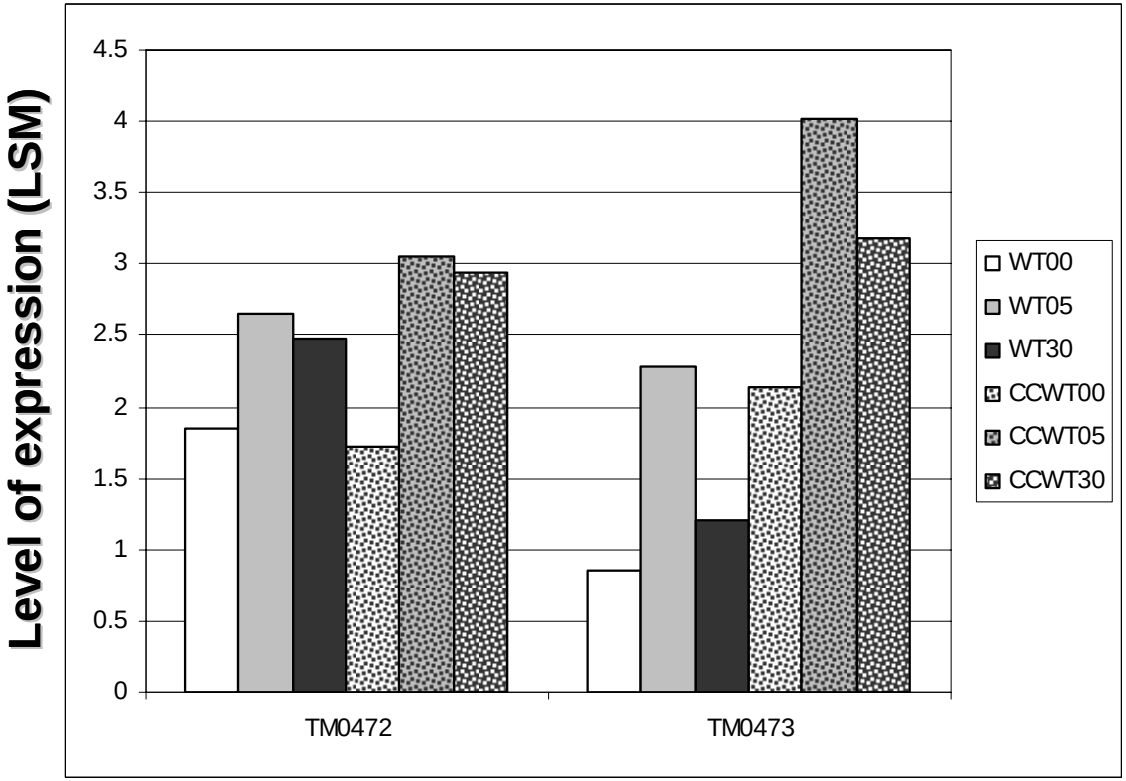


Figure 2.10. Transcriptional response of genes associated with the synthesis of pyridoxin

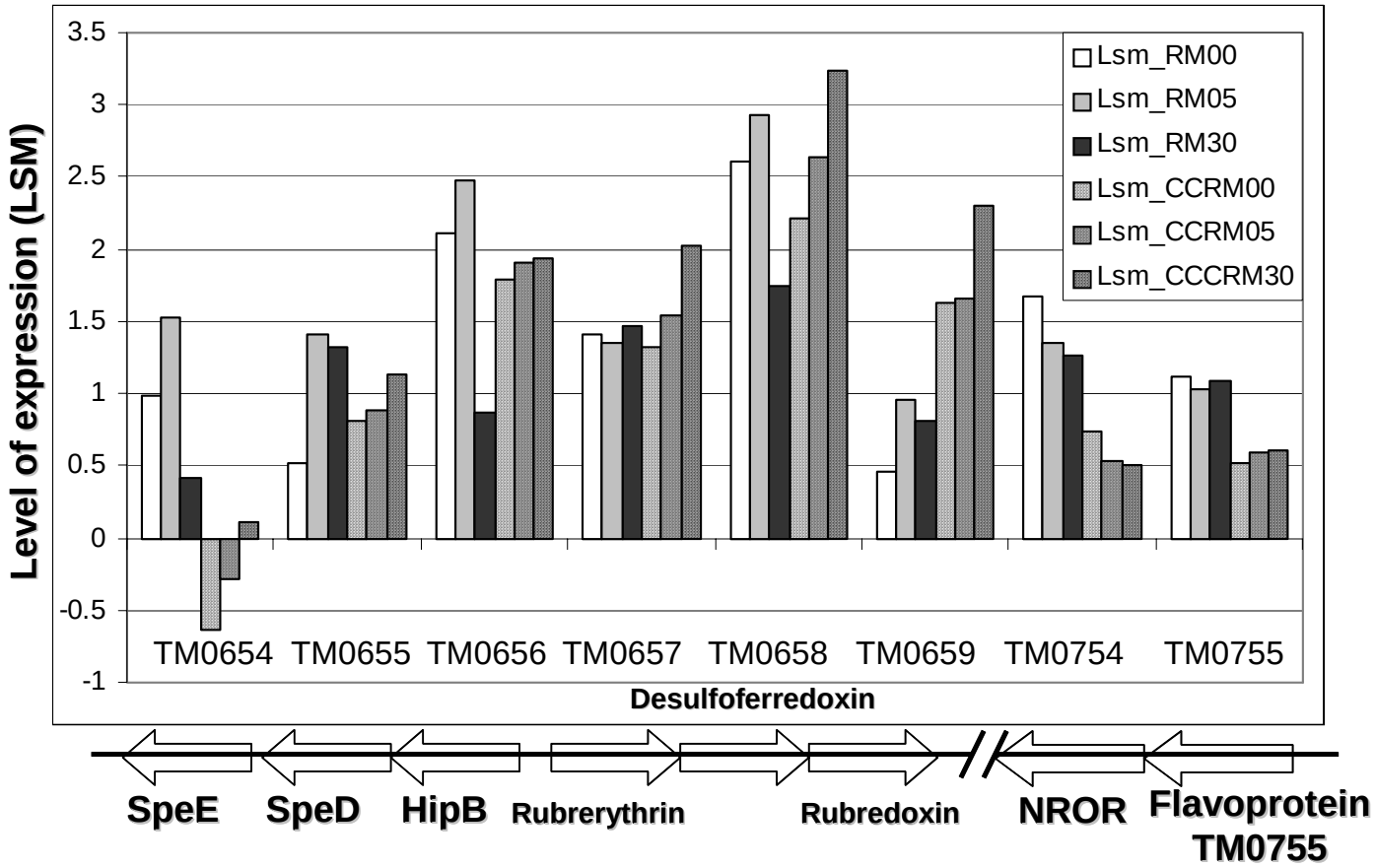


Figure 2.11. Transcriptional profile of genes associated to spermidine synthase in *T. maritima* RM strain: The present figure provides the relative levels of expression of the genes located between TM0654 and TM0659 and genes related to the superoxide reductase ROS detoxification pathway previously described in *P. furiosus* (42). CCRM00, CCRM05 and CCRM30 corresponds to conditions in continuous culture

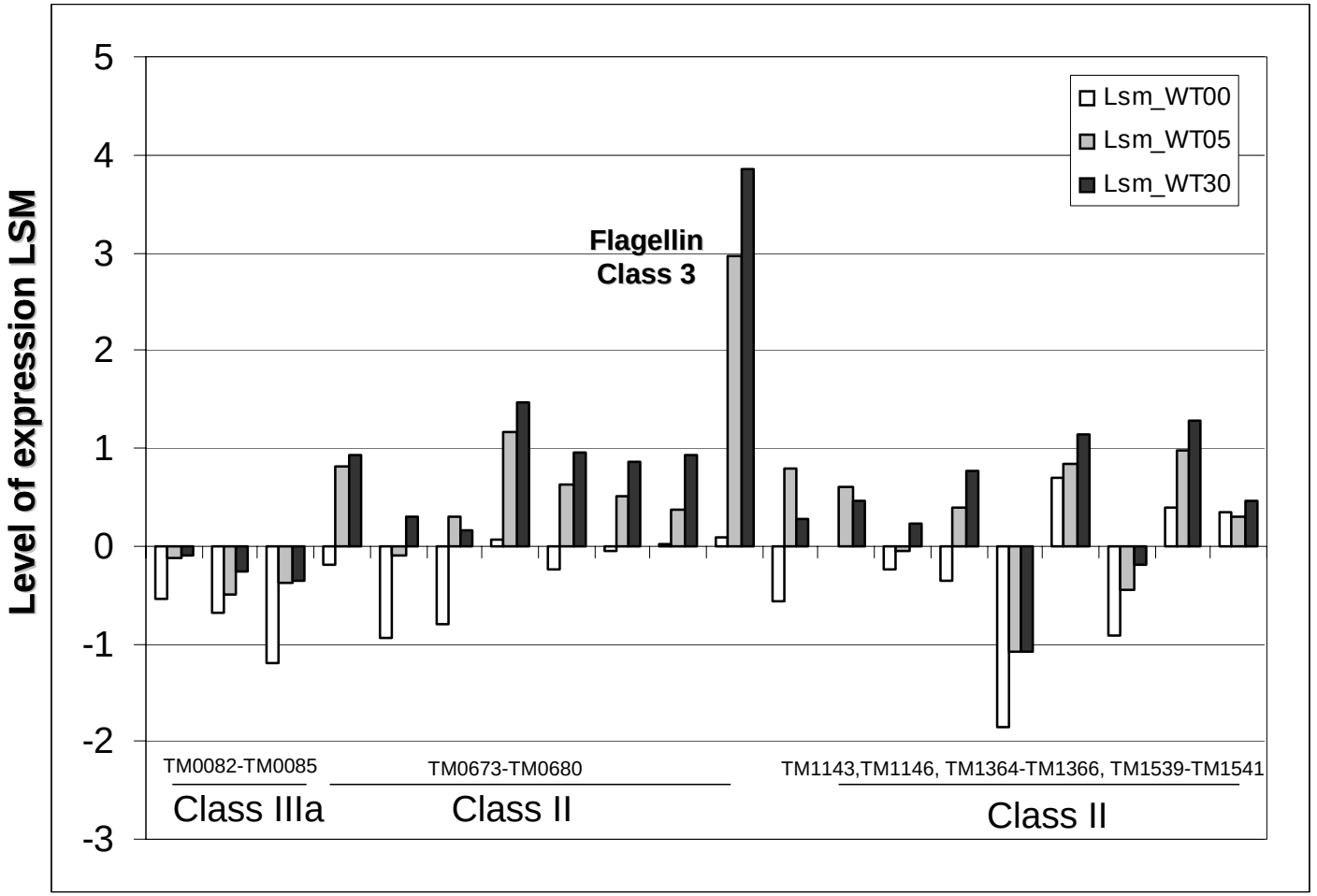


Figure 2.12: Flagellar genes up-regulated in the wild type strain upon exposure to CAM. The genes over-expressed were the following: TM0082 (FlgL), TM0083 (FlgK), TM0085 (FlgM), TM0673 (FlgD), TM0675 (FlgE), TM0677 (FlgE), TM0678 (FliL), TM0679 (FliM), TM0680 (FliY), TM0758 (Flagellin, FlgL), TM1143 (methyl accepting chemotaxis protein), TM1146 (methyl accepting chemotaxis protein), TM1364 (FlgB), TM1365 (FlgC), TM1366 (FliE), TM1539 (P-ring protein), TM1541 (P-ring protein), TM1833 (Methyl accepting chemotaxis protein).

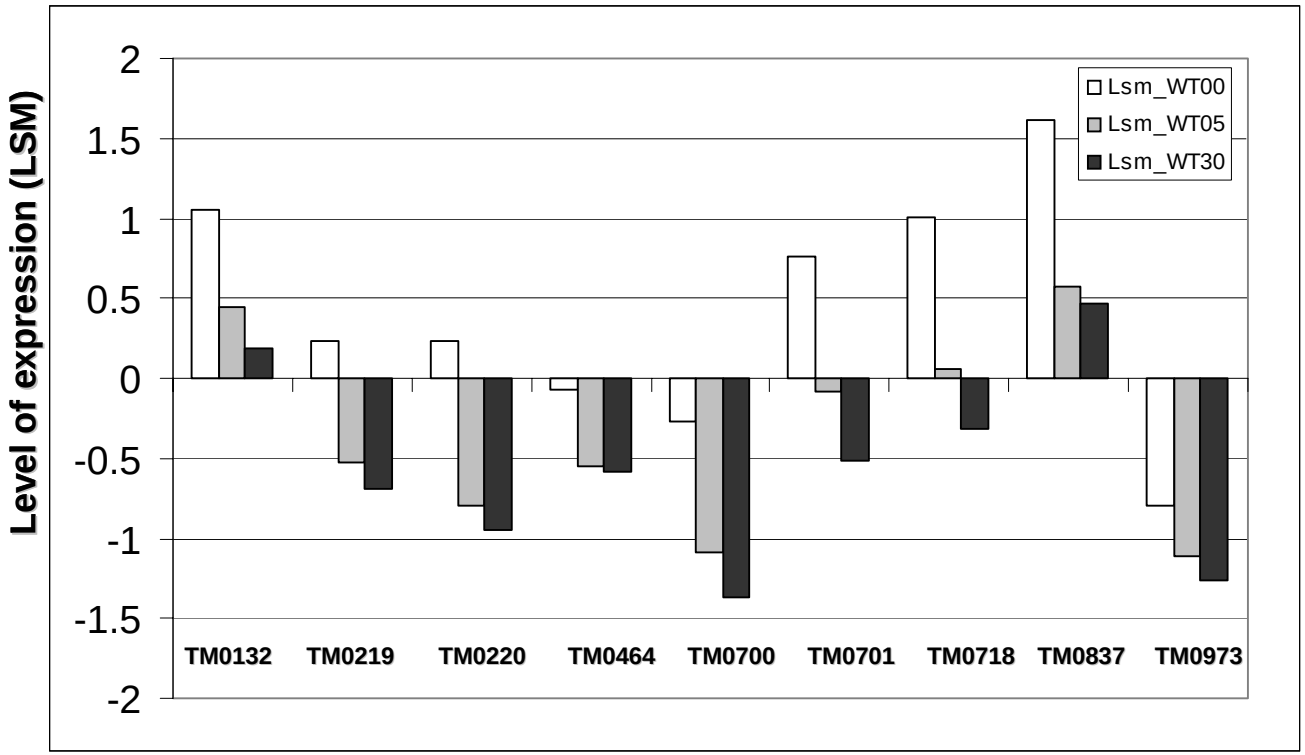


Figure 2.13: Flagellar genes down-regulated in the wild type strain upon exposure to CAM : All the genes presented in this figure correspond to class II flagellar genes. The genes down-regulated were the following: TM0132 (Flagellin FglL), TM0219 (FliH), TM0220 (FliG), TM0464 (Chemotactic methyltransferase), TM0700 (CheY), TM0701 (CheW), TM0718 (purine binding chemotaxis protein), TM0837 (CheW), TM0937 (Methyl-accepting chemoreceptor).

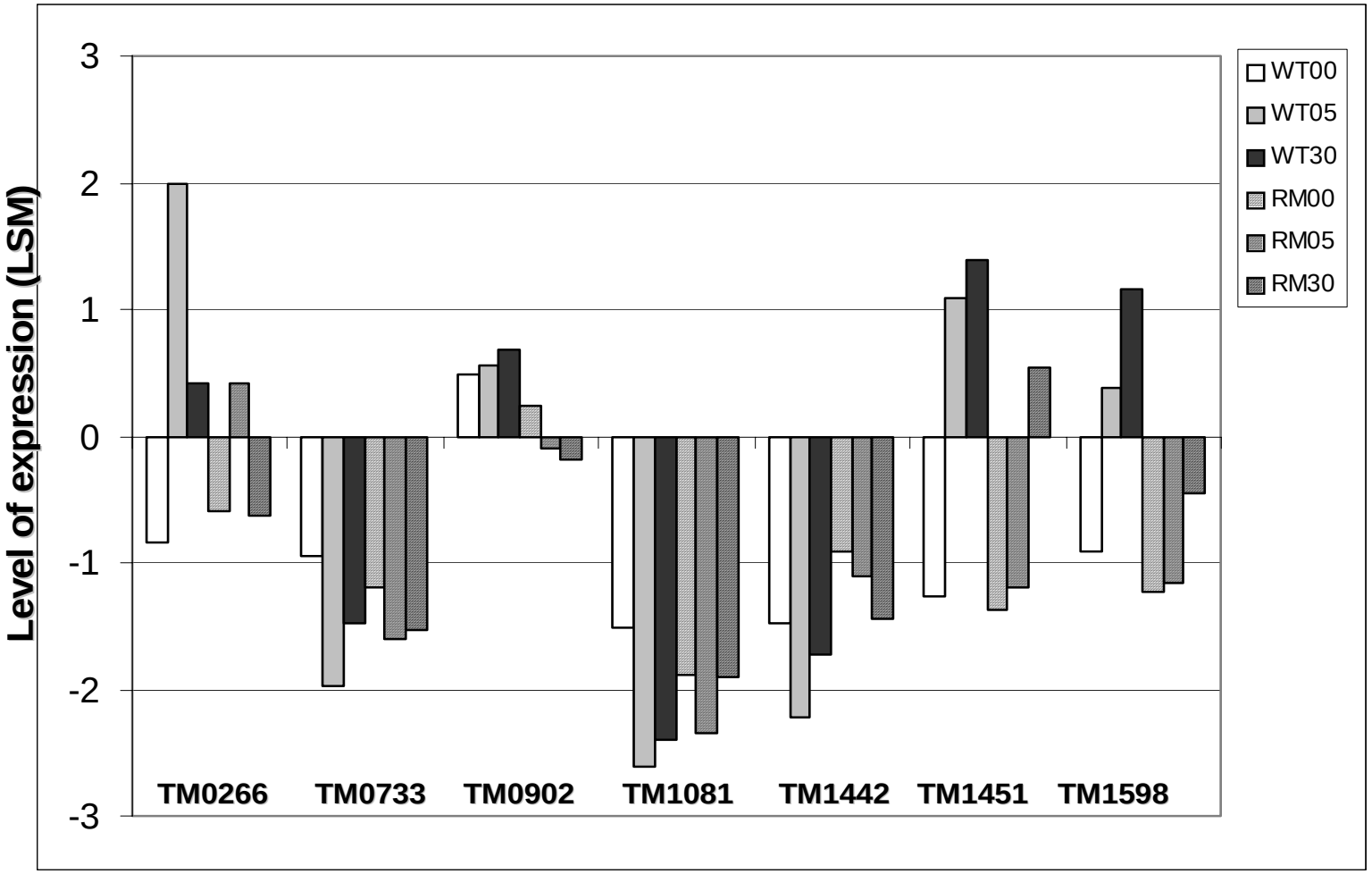


Figure 2.14: Response of global regulatory genes in WT and RM *T. maritima* upon exposure to CAM in batch culture conditions: TM0266 (HU-DNA binding protein), TM0733, anti-sigma factor, TM0902 (σ^{28}), TM1081 Anti-sigma factor, TM1442 Anti-sigma factor, TM1451 (σ^{A}), TM1598 (σ^{E}). Regulatory factor (σ^{H}) TM0534 was excluded because it did not present differential transcription.

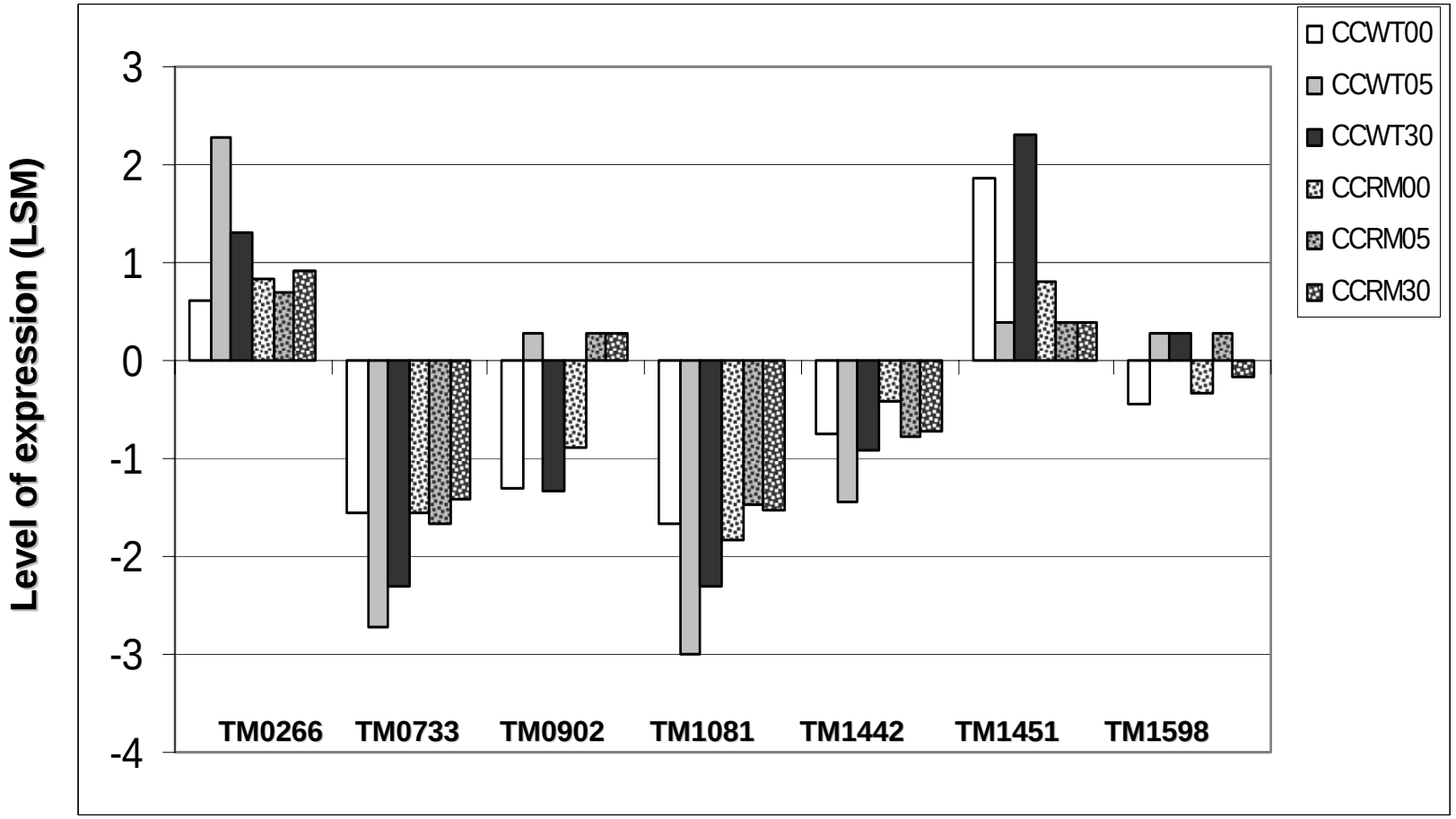


Figure 2.15: Response of some global regulatory genes in *T. maritima,* wild type and mutant strain upon exposure to CAM in continuous culture conditions. TM0266 (HU-DNA binding protein), TM0733, anti-sigma factor, TM0902 (σ^{28}), TM1081Anti-sigma factor, TM1442 Anti-sigma factor, TM1451 (σ^{A}), TM1598 (σ^{E}). Regulatory factor (σ^{H}) TM0534 was excluded because it did not present differential transcription.

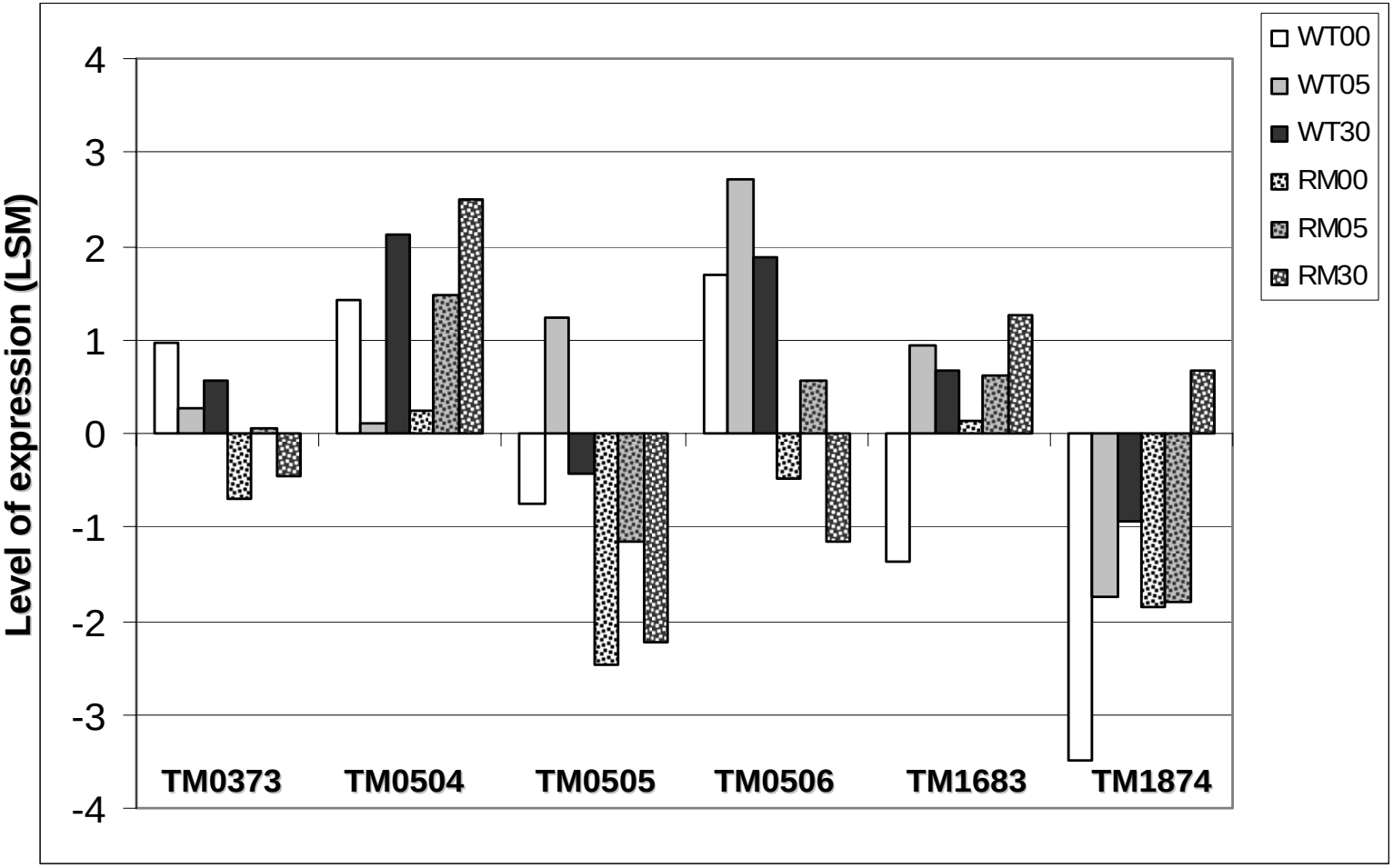


Figure 2.16: Induction of TM0504, cold shock, and Heat shock proteins *T. maritima*, wild type and mutant strain upon exposure to CAM in Batch culture. TM0373 (DnaK), TM0504, TM0505 (GroES) TM0506 (GroEL), TM1683 (CspC), TM1874 (CspC)

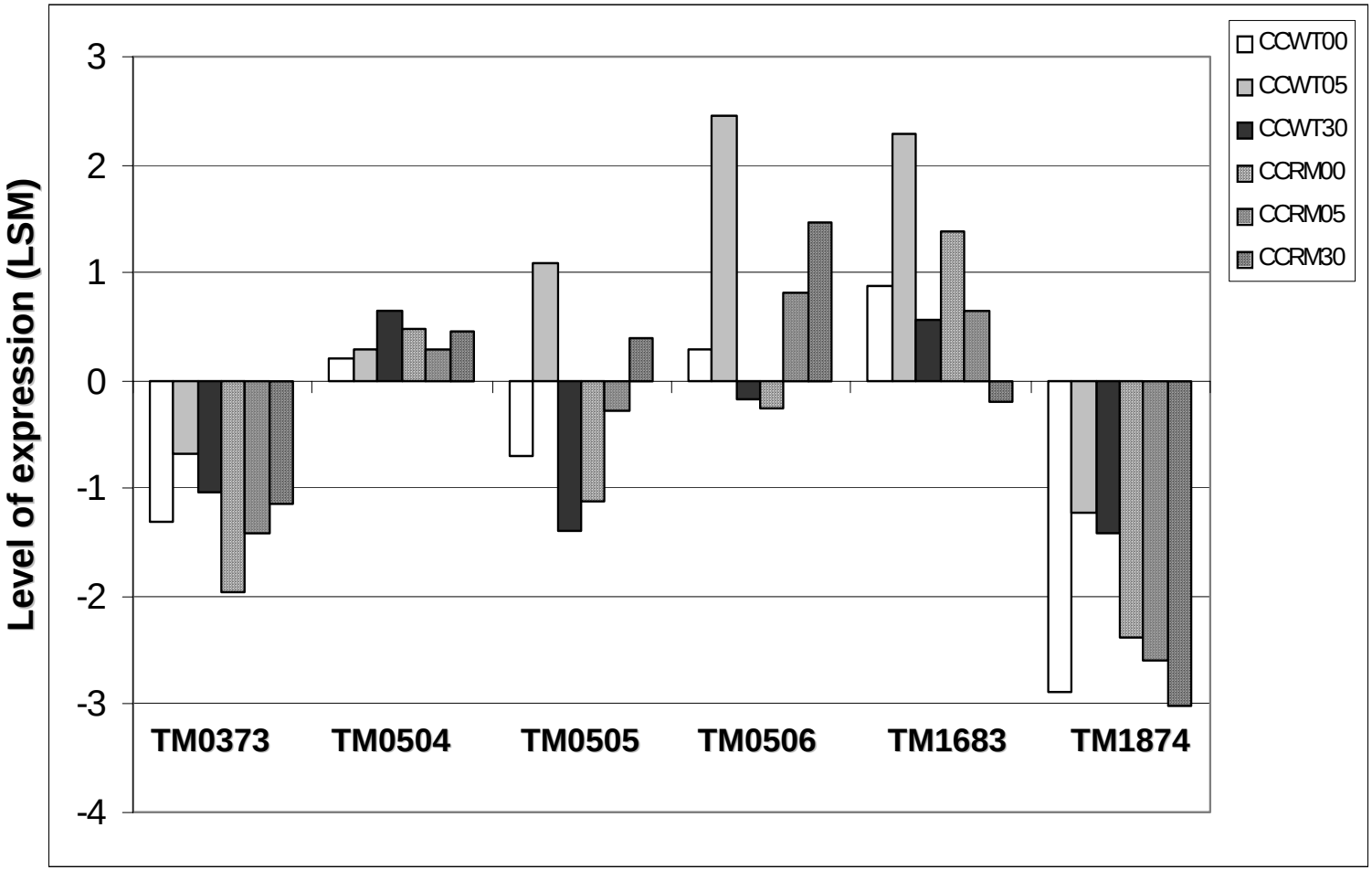


Figure 2.17: Induction of TM0504, cold shock, and Heat shock proteins *T. maritima,* wild type and mutant strain upon exposure to CAM in continuous culture conditions: TM0373 (DnaK), TM0504, TM0505 (GroES) TM0506 (GroEL), TM1683 (CspC), TM1874 (CspC).

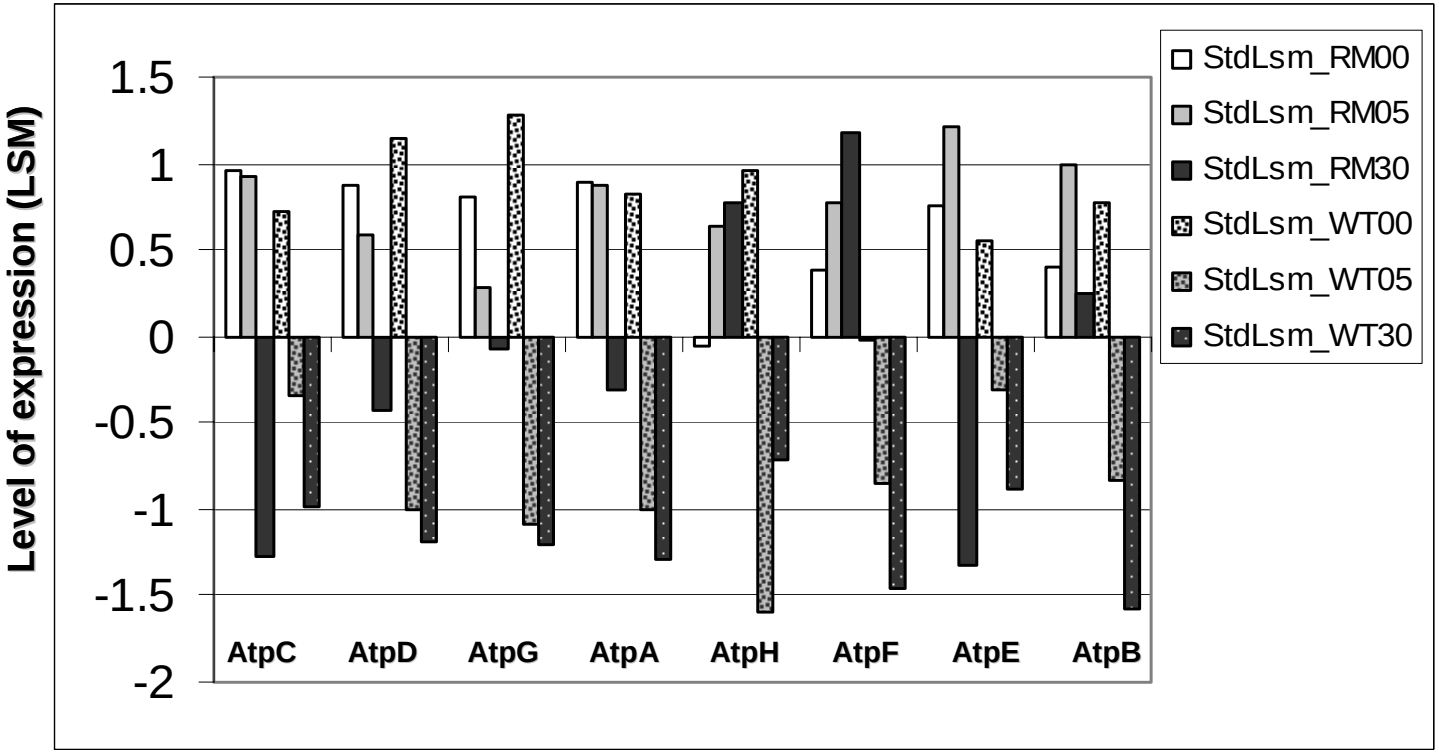


Figure 2.18: Down-regulation of components of the F1/FO ATP synthase. AtpC (TM1609), AtpD (TM1610), AtpG (TM1611) AtpA (TM1612), AtpH (TM1613), AtpF (TM1614), AtpE (TM1615), AtpB (TM1616). Although not as consistent when compared to the pattern of expression on the wild type strain, down-regulation of the ATP synthase genes is observed in the mutant strain after 30min of treatment with CAM.

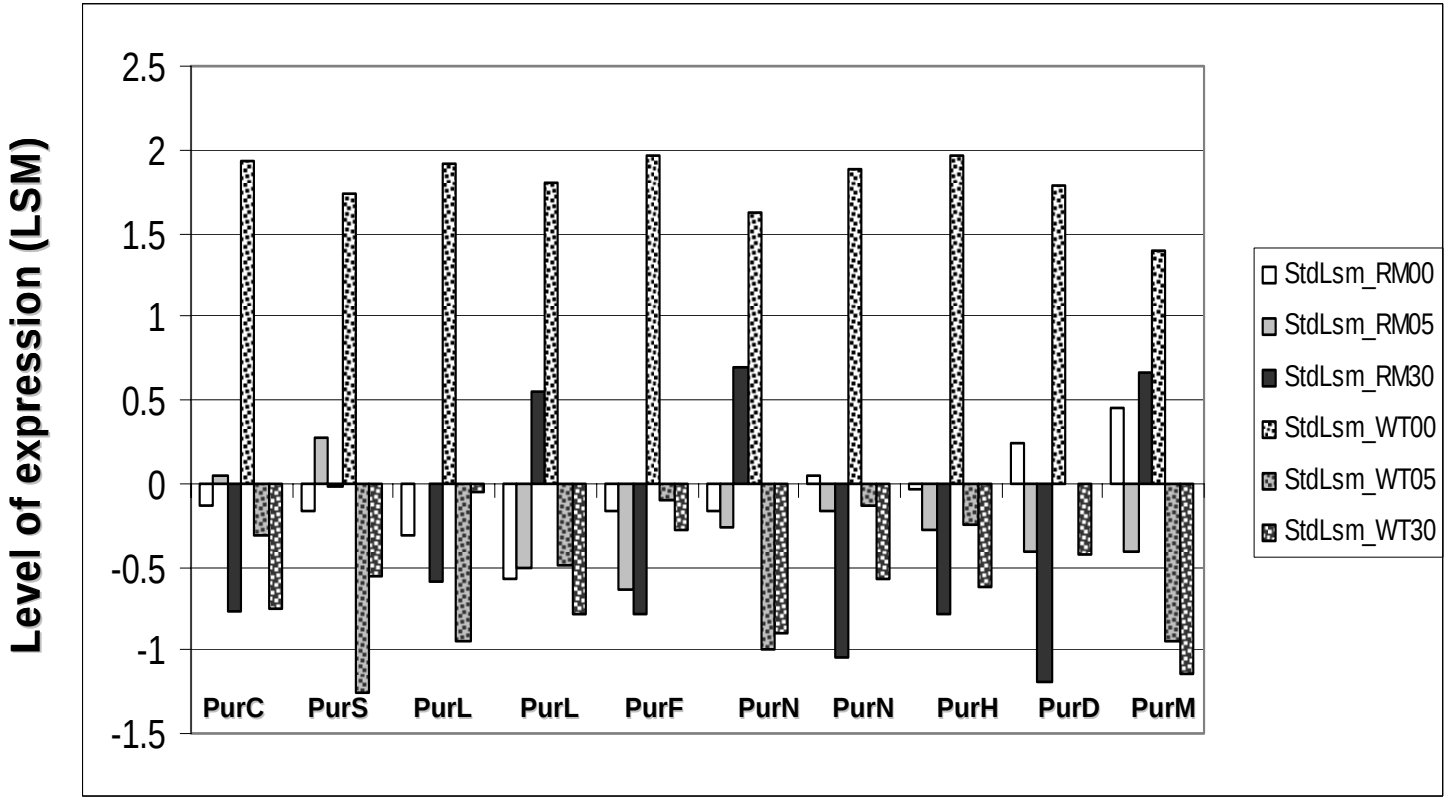


Figure 2.19: Down-regulation of some components of the *de novo* purine synthesis pathway of *T. maritima*. PurC (TM1243), PurS (TM1245), PurL (TM1245), PurL (TM1246), PurF (TM1247), PurN (TM1248), PurH (TM1249), PurD (TM1250), PurM (TM1251). Although not as consistent when compared to the pattern of expression on the wild type strain, down-regulation of the ATP synthase genes is observed in the mutant strain after 30 min of treatment with CAM.

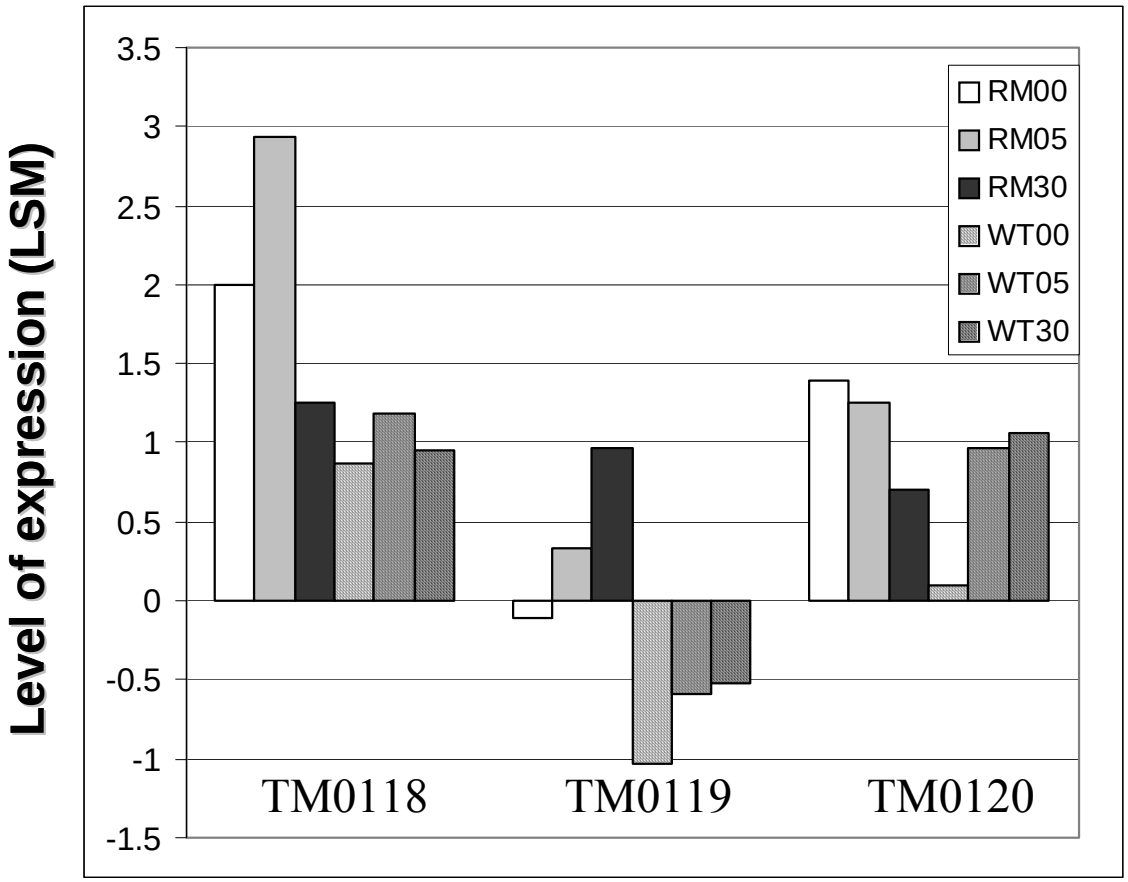


Figure 2.20: Level of expression of TM0119 a putative acetamidase/formamidase transcriptionally responsive to CAM challenge .

Table 2.1. Summary of differentially expressed ORFs in Batch and continuous culture experiments of CAM challenge

	Up-regulated	Down-regulated	Total
BATCH			
WT05 vs. WT00	139	135	274
WT30 vs. WT05	7	29	36
WT30 vs. WT00	166	186	352
RM05 vs. RM00	32	28	60
RM30 vs. RM05	279	241	520
RM30 vs. RM0	216	184	400
RM00 vs. WT00	75	56	131
RM05 vs. WT05	218	212	430
RM30 vs. WT30	151	97	227
CONTINUOUS			
WT05 vs. WT00	125	131	256
WT30 vs. WT05	32	47	79
WT30 vs. WT00	81	102	183
RM05 vs. RM00	7	1	8
RM30 vs. RM05	0	5	5
RM30 vs. RM00	23	7	30
RM00 vs. WT00	7	12	19
RM05 vs. WT05	136	135	271
RM30 vs. WT30	74	80	154

SUPPLEMENTARY MATERIAL

Response of wild-type and resistant-mutant strains of the hyperthermophilic bacterium *Thermotoga maritima* to chloramphenicol challenge

Clemente I. Montero, Matthew R. Johnson, Chung-Jung Chou, Shannon B. Conners, Sarah G. Geouge, Sabrina Tachdjian and Robert M. Kelly

SUMMARY OF DIFFERENTIALLY EXPRESSED ORFS			
	Up-regulated	**Down-regulated**	**Total**
BATCH			
WT05 vs. WT00	139	135	274
WT30 vs. WT05	7	29	36
WT30 vs. WT00	166	186	352
RM05 vs. RM00	32	28	60
RM30 vs. RM05	279	241	520
RM30 vs. RM00	216	184	400
RM00 vs. WT00	75	56	131
RM05 vs. WT05	218	212	430
RM30 vs. WT30	151	97	227
CONTINUOUS			
WT05 vs. WT00	125	131	256
WT30 vs. WT05	32	47	79
WT30 vs. WT00	81	102	183
RM05 vs. RM00	7	1	8
RM30 vs. RM05	0	5	5
RM30 vs. RM00	23	7	30
RM00 vs. WT00	7	12	19
RM05 vs. WT05	136	135	271
RM30 vs. WT30	74	80	154

TABLE 2.2. Differentially expressed ORFs for *T. maritima* batch CAM challenge RM00 vs. WT00

56 ORFs up-regulated in RM + 75 ORFs up-regulated in WT = 131 Total ORFs

Gene	Log(2) fold change RM00 vs. WT00	-log10pval	Annotation
TM0080	1.1	6.6	iron(III) ABC transporter, periplasmic-binding protein
TM0118	1.1	10.8	ribonucleotide reductase, B12-dependent
TM0121	1.3	7.8	conserved hypothetical protein
TM0122	1.1	3.7	ferric uptake regulation protein
TM0271	1	7.7	hypothetical protein
TM0380	1.1	2.2	
TM0381	1.1	4.3	dihydrolipoamide dehydrogenase
TM0384	1.9	7.3	ribonucleoside-triphosphate reductase-related protein
TM0385	1.2	7.7	conserved hypothetical protein
TM0392	1	2.3	conserved hypothetical protein
TM0403	1.3	3.8	nitrogen regulatory protein P-II
TM0454	1.8	3.7	ribosomal protein L11
TM0455	1.1	5	ribosomal protein L1
TM0456	1.9	3.4	ribosomal protein L10
TM0457	1.4	2.8	ribosomal protein L7/L12
TM0466	1	5.9	conserved hypothetical protein
TM0575	1.1	2.8	crossover junction endodeoxyribonuclease
TM0654	1	3.3	spermidine synthase
TM0656	1.8	12.2	conserved hypothetical protein
TM0767	2.4	21	maltodextrin glycosyltransferase
TM0786	1.5	12.1	hypothetical protein
TM0979	1.6	18.6	conserved hypothetical protein
TM0980	1.9	6.6	hypothetical protein
TM0982	1.4	8.9	conserved hypothetical protein
TM0983	1.5	11.8	conserved hypothetical protein
TM1059	1.1	10	spoVS-related protein
TM1125	1.1	10.1	hypothetical protein
TM1126	1.3	14.2	hypothetical protein
TM1168	2.6	17.8	
TM1263	1.2	17.5	phosphate ABC transporter, permease protein
TM1264	1.6	8.7	phosphate ABC transporter, periplasmic phosphate-binding protein
TM1276	1	23.9	sugar ABC transporter, ATP-binding protein
TM1345	1	9.1	polynucleotide phosphorylase
TM1437	1.2	5	dimethyladenosine transferase
TM1454	1	20.7	ribosomal protein L13
TM1463	1	8.7	ribonuclease P protein component
TM1469	1	14	Glucokinase
TM1471	1.3	4.9	ribosomal protein L17
TM1473	1.1	2.4	ribosomal protein S4
TM1474	1.4	4.5	ribosomal protein S11
TM1475	1.4	3.1	ribosomal protein S13

TM1477	1	5.5	
TM1478	1	14.3	methionine aminopeptidase
TM1479	1.4	9.5	adenylate kinase
TM1480	1.4	3.8	preprotein translocase SecY subunit
TM1482	1.2	3.1	ribosomal protein L30
TM1483	1.1	1.6	ribosomal protein S5
TM1488	1.6	4	ribosomal protein L5
TM1490	1.2	4	ribosomal protein L14
TM1491	1.5	8.6	ribosomal protein S17
TM1492	2.4	17.7	ribosomal protein L29
TM1493	2.3	12.7	ribosomal protein L16
TM1494	2.5	10.1	ribosomal protein S3
TM1495	1.1	1.7	ribosomal protein L22
TM1498	2.3	12.4	ribosomal protein L23
TM1499	2.4	32.6	ribosomal protein L4
TM1500	2.4	10.5	ribosomal protein L3
TM1501	2.2	16.4	ribosomal protein S10
TM1502	1.8	6.5	translation elongation factor Tu
TM1503	1.4	5.1	translation elongation factor G
TM1504	1	11.6	ribosomal protein S7
TM1505	1.3	2.3	ribosomal protein S12
TM1590	1.9	16.5	translation initiation factor IF-3
TM1591	2.2	13.3	ribosomal protein L35
TM1592	1.8	5.5	ribosomal protein L20
TM1593	1.4	4.5	conserved hypothetical protein
TM1627	1	5	general stress protein Ctc
TM1628	1	7.5	phosphoribosyl pyrophosphate synthetase
TM1658	1	7.9	S-adenosylmethionine synthetase
TM1683	1.5	10.5	cold shock protein
TM1765	1	6.2	N utilization substance protein B
TM1836	1.2	13.2	maltose ABC transporter, permease protein
TM1839	3	35.7	maltose ABC transporter, periplasmic maltose-binding protein
TM1870	1.1	8.2	septum site-determining protein MinD
TM1874	1.6	11.5	cold shock protein
TM0009	-1.5	3.5	hypothetical protein
TM0012	-1	5.4	NADP-reducing hydrogenase, subunit A
TM0295	-1.1	3.6	transaldolase-related protein
TM0373	-1.7	13.9	dnaK protein
TM0374	-1.3	6.1	heat shock protein, class I
TM0394	-1.3	3	conserved hypothetical protein
TM0395	-1.7	12.3	NADH oxidase, putative
TM0396	-1.7	18.8	iron-sulfur cluster-binding protein
TM0397	-1	12.1	glutamate synthase, alpha subunit
TM0398	-1.1	5.7	conserved hypothetical protein
TM0423	-2.2	14.8	glycerol dehydrogenase
TM0504	-1.2	12.4	hypothetical protein
TM0505	-1.7	9.9	groES protein
TM0506	-2.2	20.4	groEL protein

TM0545	-1.2	2.9	homoserine kinase, putative
TM0549	-1	3.1	acetolactate synthase, small subunit
TM0562	-1.4	15.4	hypothetical protein
TM0563	-1	9.5	conserved hypothetical protein
TM0665	-1.8	11.3	cysteine synthase
TM0666	-1.8	8.8	serine acetyltransferase
TM0714	-1.2	18	hypothetical protein
TM0753	-1.4	12.1	ubiquinone/menaquinone biosynthesis methyltransferase,
TM0754	-1.1	8.6	Oxidoreductase
TM0755	-1.8	18.6	conserved hypothetical protein
TM0849	-1.7	11	dnaJ protein
TM0850	-1.1	6.6	grpE protein, putative
TM0851	-1.1	9.3	heat shock operon repressor HrcA
TM0873	-1.3	4.7	
TM0881	-1.3	13.2	homoserine O-succinyltransferase
TM0882	-2.1	15.3	O-acetylhomoserine sulfhydrylase
TM0963	-1.1	7.4	Oligoendopeptidase, putative
TM0964	-1.1	6.6	conserved hypothetical protein
TM0965	-1.6	19.9	phosphoribosylaminoimidazole carboxylase-related protein
TM0966	-1.3	5.9	conserved hypothetical protein
TM1111	-1	7.6	hypothetical protein
TM1151	-1.4	11	oligopeptide ABC transporter, ATP-binding protein
TM1152	-1.2	7.1	oligopeptide ABC transporter, ATP-binding protein
TM1153	-1	3.4	oligopeptide ABC transporter, permease protein
TM1154	-2.2	10	oxidoreductase, sol/devB family
TM1155	-2.7	12	glucose-6-phosphate 1-dehydrogenase
TM1172	-1	10.9	prismane protein
TM1243	-1.1	9.2	phosphoribosylaminoimidazole-succinocarboxamide synthase
TM1245	-1.8	20.3	phosphoribosylformylglycinamidine synthase I
TM1246	-1.7	5.6	phosphoribosylformylglycinamidine synthase II
TM1247	-1.2	10.8	amidophosphoribosyltransferase
TM1248	-1.2	4.7	phosphoribosylglycinamide formyltransferase
TM1249	-1.4	14.2	phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
TM1369	-1.8	11	conserved hypothetical protein
TM1370	-2.9	25.4	hypothetical protein
TM1371	-2.4	26	aminotransferase, class V
TM1372	-1.7	4.6	nitrogen fixation protein NifU-related protein
TM1375	-2.4	21.5	spermidine/putrescine ABC transporter, periplasmic spermidine/putrescine-binding protein
TM1400	-2	23.4	aspartate aminotransferase, putative
TM1401	-1	5.6	D-3-phosphoglycerate dehydrogenase
TM1536	-1.1	8.3	conserved hypothetical protein
TMrrnaA16	-1.1	9.4	

TABLE 2.3. Differentially expressed ORFs for *T. maritima* batch CAM challenge RM05 vs. WT05			
212 ORFs up-regulated in RM + 218 ORFs up-regulated in WT = 430 Total ORFs			
Gene	**Log(2) fold change RM05 vs. WT05**	**-$\log_{10}$pval**	**Annotation**
TM0035	2.5	6.9	hypothetical protein
TM0039	1	1.3	conserved hypothetical protein
TM0042	1.3	6.6	aminopeptidase P, putative
TM0087	1	0.9	conserved hypothetical protein
TM0088	1.1	0.8	comE protein, putative
TM0089	1.5	1.8	hypothetical protein
TM0092	1.6	4.7	hypothetical protein
TM0093	1.5	1.1	hypothetical protein
TM0094	1.5	8.3	general secretion pathway protein F, putative
TM0097	1.2	0.7	conserved hypothetical protein
TM0108	1.6	0.8	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
TM0118	1.8	9.9	ribonucleotide reductase, B12-dependent
TM0137	2	3.4	tryptophan synthase, alpha subunit
TM0138	1	1.9	tryptophan synthase, beta subunit
TM0139	1.3	4.8	phosphoribosylanthranilate isomerase
TM0140	1.6	1.7	indole-3-glycerol phosphate synthase
TM0141	1.5	10.9	anthranilate synthase component II
TM0146	1.2	4.1	ATP-dependent Clp protease, ATPase subunit clpX
TM0147	1.3	5.2	conserved hypothetical protein
TM0148	1.9	9.8	glucosamine--fructose-6-phosphate aminotransferase, isomerizing
TM0149	2.2	13.8	fatty acid/phospholipid synthesis protein
TM0158	1	2	prolipoprotein diacylglyceryl transferase
TM0164	1	0.7	conserved hypothetical protein
TM0165	1.1	0.7	Holliday junction DNA helicase
TM0168	1	1.9	leucyl-tRNA synthetase
TM0169	1.1	1.6	conserved hypothetical protein
TM0172	1.4	0.6	adenosylhomocysteinase
TM0174	1.4	1.1	pyrophosphatase, proton-translocating
TM0188	1.3	6.5	conserved hypothetical protein
TM0189	1.1	1	iron(III) ABC transporter, periplasmic iron-binding protein, putative
TM0190	1.4	1	iron(III) ABC transporter, permease protein, putative
TM0191	1.1	0.8	iron(III) ABC transporter, ATP-binding protein, putative
TM0203	1	0.6	ABC transporter, permease protein, cysTW family
TM0204	1.3	1.3	ABC transporter, ATP-binding protein
TM0205	1.3	0.7	ATP-dependent DNA helicase
TM0206	1.4	0.7	hypoxanthine phosphoribosyltransferase
TM0208	1.1	1	pyruvate kinase
TM0212	1.5	1.6	glycine cleavage system H protein
TM0219	1.1	2.4	flagellar export/assembly protein
TM0220	1.2	6.3	flagellar motor switch protein FliG
TM0231	1.5	7	UDP-N-acetylmuramate-alanine ligase

TM0240	1.2	0.8	glucose-1-phosphate adenylyltransferase
TM0257	1.4	1.9	
TM0258	1	1.8	DNA topoisomerase
TM0261	1.3	0.9	phosphate permease, putative
TM0265	1	0.7	excinuclease ABC, subunit C
TM0271	1.5	4.3	hypothetical protein
TM0272	1.2	7.5	pyruvate,orthophosphate dikinase
TM0273	1	3.6	fructose-bisphosphate aldolase
TM0290	1.4	1.8	citrate synthase
TM0293	1.4	1.3	gamma-glutamyl phosphate reductase
TM0296	1	0.7	fructokinase
TM0312	2.2	3.5	conserved hypothetical protein
TM0313	2.3	11.4	K+ channel, beta subunit
TM0372	1.4	0.7	cation efflux system protein, putative
TM0380	2.1	1.3	
TM0384	3.1	3.3	anaerobic ribonucleoside-triphosphate reductase-related protein
TM0385	2.2	7.8	conserved hypothetical protein
TM0386	1.4	4.4	bacterioferritin comigratory protein/NADH dehydrogenase
TM0393	2.5	10.3	transcriptional regulator, XylR-related
TM0394	1.1	0.7	conserved hypothetical protein
TM0397	1.3	6.4	glutamate synthase, alpha subunit
TM0403	1	0.5	nitrogen regulatory protein P-II
TM0452	1	2.3	preprotein translocase SecE subunit
TM0453	1.1	0.4	N utilization substance protein G
TM0454	3	1.9	ribosomal protein L11
TM0456	2.6	1.2	ribosomal protein L10
TM0457	1.4	1.2	ribosomal protein L7/L12
TM0458	1.5	13.1	DNA-directed RNA polymerase, beta subunit
TM0459	1.2	3.1	DNA-directed RNA polymerase, beta' subunit
TM0463	1.4	1.4	lipoprotein signal peptidase
TM0466	1.6	4.4	conserved hypothetical protein
TM0471	1.4	4.3	hypothetical protein
TM0478	1.5	4.4	tyrosyl-tRNA synthetase
TM0479	1.1	1	hypothetical protein
TM0488	1.2	1.2	hemK protein
TM0496	1.1	8.9	DNA polymerase III, epsilon subunit, putative
TM0499	1.2	1.2	hypothetical protein
TM0501	1.6	3.1	oligopeptide ABC transporter, ATP-binding protein
TM0502	1.4	1.1	oligopeptide ABC transporter, permease protein
TM0504	1.4	3.6	hypothetical protein
TM0509	1	5.8	UDP-glucose 4-epimerase, putative
TM0513	1	2.4	comM protein
TM0514	1.6	1.1	prolyl-tRNA synthetase
TM0523	1	3.7	hypothetical protein
TM0542	1	3.2	malate oxidoreductase
TM0544	1.6	1	ABC transporter, ATP-binding protein
TM0545	1.8	1.7	homoserine kinase, putative
TM0546	1.4	0.7	threonine synthase

TM0550	1.7	8	ketol-acid reductoisomerase
TM0555	1.1	1.8	3-isopropylmalate dehydratase, small subunit
TM0567	1	0.9	conserved hypothetical protein
TM0575	2.1	1.6	crossover junction endodeoxyribonuclease
TM0576	1.7	5.4	DNA polymerase III, alpha subunit
TM0580	1.1	3	cell division protein FtsH
TM0581	1.4	1.7	hypothetical protein
TM0585	1	1.2	lipopolysaccharide biosynthesis protein BplA
TM0626	1	2.1	hypothetical protein
TM0635	1.3	1.8	hypothetical protein
TM0636	1.2	1.6	hypothetical protein
TM0684	1.1	1	hypothetical protein
TM0687	1	0.7	conserved hypothetical protein
TM0700	1.3	3.1	chemotaxis response regulator CheY
TM0701	1.3	4	purine-binding chemotaxis protein
TM0712	1	2.5	conserved hypothetical protein
TM0713	1	0.9	hypothetical protein
TM0717	1.2	6.2	propionyl-CoA carboxylase, gamma subunit
TM0718	1	1.9	purine-binding chemotaxis protein
TM0720	1.3	0.7	serine hydroxymethyltransferase
TM0722	1.2	2.1	vacB protein
TM0746	1.2	2.8	hypothetical protein
TM0767	1.4	3.2	maltodextrin glycosyltransferase
TM0804	2	4	conserved hypothetical protein
TM0827	1.1	1.8	ABC transporter, ATP-binding protein, putative
TM0831	1.6	1.3	branched-chain amino acid aminotransferase, putative
TM0833	1.1	2.4	DNA gyrase, subunit B
TM0859	1.6	2	hypothetical protein
TM0861	1.2	2.3	protein-export membrane protein SecF, putative
TM0862	1	1.9	glucose-1-phosphate thymidylyltransferase
TM0868	1.1	4.3	glutaredoxin-related protein
TM0872	1.2	2.5	conserved hypothetical protein
TM0893	1.1	4.2	bacitracin resistance protein
TM0896	1	0.7	galactose-1-phosphate uridylyltransferase, putative
TM0979	1.2	3.9	conserved hypothetical protein
TM0980	1.4	1	hypothetical protein
TM1078	1.1	1.6	conserved hypothetical protein
TM1084	1	7.9	DNA gyrase, subunit A
TM1125	1.1	2.6	hypothetical protein
TM1148	1.7	7.1	isocitrate dehydrogenase
TM1168	2.1	4.7	
TM1220	1.5	4.9	oligopeptide ABC transporter, ATP-binding protein
TM1239	1.8	3.7	conserved hypothetical protein
TM1240	1.3	1.8	conserved hypothetical protein
TM1255	1.4	1.8	aspartate aminotransferase
TM1259	1.2	1.9	phosphate regulon transcriptional regulatory protein PhoB
TM1264	1.7	2.4	phosphate ABC transporter, periplasmic phosphate-binding protein
TM1267	3	4.2	thiH protein, putative

TM1269	1.6	1.4	biotin synthetase, putative
TM1273	1.6	5.8	glutamyl tRNA-Gln amidotransferase, subunit B
TM1274	1.4	2.2	hypothetical protein
TM1286	2.8	14.4	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
TM1345	2.6	10	polynucleotide phosphorylase
TM1347	1.8	1.1	inosine-5'-monophosphate dehydrogenase
TM1362	2	1.4	motility protein PilT
TM1363	1.4	2.5	peptide chain release factor RF-1
TM1381	1.3	1.6	hypothetical protein
TM1383	1.6	2.1	conserved hypothetical protein
TM1442	1.1	1.7	anti-sigma factor antagonist, putative
TM1445	1.2	4.1	
TM1447	1.2	2.5	conserved hypothetical protein
TM1449	1.1	0.9	conserved hypothetical protein
TM1459	1.8	2.7	conserved hypothetical protein
TM1461	1.5	2.9	inner membrane protein, putative
TM1463	1	2.5	ribonuclease P protein component
TM1469	1.3	7.5	glucokinase
TM1470	1.4	9.3	transcription termination factor Rho
TM1471	1.3	1	ribosomal protein L17
TM1473	1.1	1.9	ribosomal protein S4
TM1474	1.1	0.7	ribosomal protein S11
TM1475	1.4	1.5	ribosomal protein S13
TM1479	2.2	4.5	adenylate kinase
TM1480	2.1	1.6	preprotein translocase SecY subunit
TM1481	1.4	2.6	ribosomal protein L15
TM1482	1.6	1.1	ribosomal protein L30
TM1483	1.1	0.4	ribosomal protein S5
TM1493	1.2	1.1	ribosomal protein L16
TM1494	1.3	1	ribosomal protein S3
TM1498	1.7	1.9	ribosomal protein L23
TM1499	1.4	10.2	ribosomal protein L4
TM1500	2.2	7.3	ribosomal protein L3
TM1501	2.3	4.9	ribosomal protein S10
TM1502	1.3	0.8	translation elongation factor Tu
TM1503	1.6	1.7	translation elongation factor G
TM1505	1.7	0.8	ribosomal protein S12
TM1506	1.4	0.8	conserved hypothetical protein
TM1512	1.4	0.8	sun protein
TM1522	1.6	1.1	diaminopimelate epimerase
TM1562	1.1	2.5	hypothetical protein
TM1565	1	3.2	signal recognition particle protein
TM1566	1.1	0.6	ribosomal protein S16
TM1571	1.7	1	ribosomal protein L19
TM1590	1.2	2.1	translation initiation factor IF-3
TM1591	1.6	2.1	ribosomal protein L35
TM1592	2.2	2.4	ribosomal protein L20
TM1593	1.5	1.3	conserved hypothetical protein

TM1595	1.4	2.6	conserved hypothetical protein
TM1596	1	1.7	purine nucleoside phosphorylase
TM1607	1.4	1.1	conserved hypothetical protein
TM1610	1.3	4.3	ATP synthase F1, subunit beta
TM1611	1.2	2.3	ATP synthase F1, subunit gamma
TM1612	2.1	5	ATP synthase F1, subunit alpha
TM1615	1.6	0.8	ATP synthase F0, subunit c
TM1618	1.4	1.8	cheX protein
TM1625	1	2.3	hypothetical protein
TM1628	2.3	6.8	phosphoribosyl pyrophosphate synthetase
TM1629	1	1.7	UDP-N-acetylglucosamine pyrophosphorylase
TM1631	1	1	conserved hypothetical protein
TM1646	1	1.4	conserved hypothetical protein
TM1651	1.1	4.3	translation elongation factor G
TM1657	1.3	3.1	ribosomal protein S20
TM1658	2.2	5.9	S-adenosylmethionine synthetase
TM1677	1	2.8	transposase, putative
TM1685	1.9	14.2	conserved hypothetical protein
TM1688	1.3	2.6	hypothetical protein
TM1689	1.3	5.3	guanylate kinase
TM1691	1.9	1.6	conserved hypothetical protein
TM1704	2.2	1.6	hypothetical protein
TM1705	1.9	5.6	lysyl-tRNA synthetase
TM1715	1.5	4.6	conserved hypothetical protein
TM1767	2.3	2.1	methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase
TM1809	1.3	1.2	conserved hypothetical protein
TM1813	1	1.5	hypothetical protein
TM1832	1.1	1.7	transposase
TM1836	1.1	4	maltose ABC transporter, permease protein
TM1839	1.8	10.4	maltose ABC transporter, periplasmic maltose-binding protein
TM1870	1	1.6	septum site-determining protein MinD
TM1871	1.2	1.3	hypothetical protein
TM1872	1.3	7	conserved hypothetical protein
TM0002	-1	1.7	hypothetical protein
TM0004	-1	2.1	hypothetical protein
TM0022	-1.3	1.2	DNA mismatch repair protein
TM0025	-1	1.7	beta-glucosidase
TM0031	-1	0.5	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0032	-1	4.5	transcriptional regulator, XylR-related
TM0044	-1.4	1	hypothetical protein
TM0055	-1.3	7.1	alpha-glucuronidase
TM0059	-1.9	5.6	oligopeptide ABC transporter, permease protein
TM0060	-1.8	4.6	oligopeptide ABC transporter, permease protein
TM0061	-1.6	2.8	endo-1,4-beta-xylanase A
TM0062	-1.6	3.8	hypothetical protein
TM0064	-1.1	6.9	uronate isomerase, putative

TM0065	-1.2	1.3	transcriptional regulator, IclR family
TM0066	-1.4	3.7	2-dehydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate aldolase
TM0069	-1.3	2.5	D-mannonate hydrolase
TM0070	-1.1	2.8	endo-1,4-beta-xylanase B
TM0071	-1.4	0.9	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0086	-1.3	4.4	virulence factor MviN-related protein
TM0113	-1.1	2.9	xylU-related protein
TM0117	-1	1.4	conserved hypothetical protein
TM0167	-1.2	1.9	phosphopentomutase
TM0187	-1	2.3	
TM0198	-1.2	1.3	ATP-dependent Clp protease, ATPase subunit
TM0266	-1.6	1.4	DNA-binding protein, HU
TM0270	-1.3	5.7	conserved hypothetical protein
TM0281	-1.7	6.9	alpha-L-arabinofuranosidase
TM0295	-1.2	1	transaldolase-related protein
TM0301	-1	1.2	oligopeptide ABC transporter, permease protein
TM0304	-1.4	4.5	oligopeptide ABC transporter, ATP-binding protein
TM0305	-1.8	3	endoglucanase, putative
TM0308	-1.3	2.5	alpha-xylosidase
TM0310	-1.3	1.3	beta-D-galactosidase
TM0320	-1.1	1.7	heavy metal binding protein
TM0321	-1.4	4.6	hypothetical protein
TM0324	-1.5	1.2	conserved hypothetical protein
TM0333	-1.2	2.1	dihydroorotate dehydrogenase
TM0334	-2	1.8	dihydroorotate dehydrogenase electron transfer protein
TM0338	-1.6	2.6	hypothetical protein
TM0352	-1.2	2.8	ABC transporter, ATP-binding protein
TM0369	-2.3	5.7	conserved hypothetical protein
TM0370	-1	1.6	conserved hypothetical protein
TM0376	-1	2.2	conserved hypothetical protein
TM0411	-1.6	5	transcriptional regulator, XylR-related
TM0415	-1.2	1.3	hypothetical protein
TM0417	-1	1.7	conserved hypothetical protein
TM0423	-1.2	2.4	glycerol dehydrogenase
TM0431	-1.6	2.6	sugar ABC transporter, permease protein
TM0432	-2	6.8	sugar ABC transporter, periplasmic sugar-binding protein, putative
TM0433	-1.6	2.8	pectate lyase
TM0434	-1.7	6.6	alpha-glucosidase, putative
TM0435	-1.9	4.6	acetyl xylan esterase-related protein
TM0436	-1.6	4.6	alcohol dehydrogenase, zinc-containing
TM0437	-2	1	exo-poly-alpha-D-galacturonosidase, putative
TM0438	-1.8	9.4	6-phosphogluconate dehydrogenase, decarboxylating
TM0440	-1.3	3.9	hypothetical protein
TM0441	-1.8	4.4	oxidoreductase, short chain dehydrogenase/reductase family
TM0442	-1.6	3.3	conserved hypothetical protein
TM0443	-1.2	4.3	gluconate kinase

TM0445	-1.8	4.4	conserved hypothetical protein
TM0505	-2.4	4.1	groES protein
TM0506	-2.1	5.6	groEL protein
TM0559	-1	1.6	conserved hypothetical protein
TM0562	-2.5	18.2	hypothetical protein
TM0571	-2.1	5.9	heat shock serine protease, periplasmic
TM0589	-1	1.5	hypothetical protein
TM0602	-1	1.7	iron-dependent transcriptional repressor, putative
TM0603	-1.5	1	ribosomal protein S6
TM0607	-1	2.8	hypothetical protein
TM0638	-1	3.6	polysaccharide export protein, putative
TM0640	-1.1	2.8	conserved hypothetical protein
TM0651	-1.5	3.2	conserved hypothetical protein
TM0654	-1.1	1.7	spermidine synthase
TM0655	-1.4	5.1	conserved hypothetical protein
TM0657	-1.6	1.2	rubrerythrin
TM0681	-1.2	2.1	dehydrase-related protein
TM0688	-1.1	2	glyceraldehyde-3-phosphate dehydrogenase
TM0706	-1.2	3.6	hypothetical protein
TM0710	-1	1.2	transcriptional regulator, MarR family
TM0755	-1.3	4.3	conserved hypothetical protein
TM0757	-1.3	3.5	hypothetical protein
TM0758	-3	22.1	flagellin
TM0778	-1.4	2.8	hypothetical protein
TM0784	-2.2	12.4	hypothetical protein
TM0787	-1.3	2.9	thiamine biosynthetic enzyme
TM0788	-1.2	2.8	thiamine biosynthesis protein, putative
TM0789	-1	1.3	hypothetical protein
TM0808	-1.5	11.6	transcriptional regulator, XylR-related
TM0810	-1.5	12.1	sugar ABC transporter, periplasmic sugar-binding protein
TM0814	-1.2	2.8	N-acetylglucosamine-6-phosphate deacetylase
TM0816	-1.5	4.4	transcriptional regulator, putative, Mar family
TM0818	-1	4.1	lipopolysaccharide biosynthesis protein, putative
TM0823	-1.3	3.4	transcriptional regulator, TetR family
TM0840	-1.1	2.6	hypothetical protein
TM0849	-1	1.6	dnaJ protein
TM0850	-1.5	3.5	grpE protein, putative
TM0851	-1.3	4.1	heat shock operon repressor HrcA
TM0882	-2	3.4	O-acetylhomoserine sulfhydrylase
TM0888	-1	2.7	conserved hypothetical protein
TM0899	-1.3	1	hypothetical protein
TM0927	-1.3	4.9	ferredoxin
TM0934	-1.1	1.7	conserved hypothetical protein
TM0959	-1	2.5	ribose ABC transporter, membrane-associated protein
TM0960	-1	6.5	ribokinase
TM0963	-1.1	1.9	oligoendopeptidase, putative
TM0967	-1	3.1	integrase-recombinase protein
TM0984	-1	2	hypothetical protein
TM0985	-1.5	6.2	hypothetical protein

TM0986	-1	1.9	conserved hypothetical protein
TM0989	-1.4	9.1	conserved hypothetical protein
TM1001	-1.2	1.9	conserved hypothetical protein
TM1016	-2	3.5	hypothetical protein
TM1017	-1.5	3.7	conserved hypothetical protein
TM1030	-1.3	3	transcriptional regulator, TetR family
TM1033	-1.1	1.5	mannose-1-phosphate guanylyltransferase
TM1035	-1.1	0.9	phosphoribosyl-AMP cyclohydrolase / phosphoribosyl-ATP pyrophosphohydrolase
TM1061	-1	3.6	hypothetical protein
TM1064	-1.5	1.2	oligopeptide ABC transporter, ATP-binding protein
TM1068	-1.4	5.2	alpha-glucosidase, putative
TM1069	-1.1	2.2	transcriptional regulator, DeoR family
TM1091	-1	1.5	hypothetical protein
TM1105	-1	1.4	NADH dehydrogenase, putative
TM1112	-1.6	2.1	hypothetical protein
TM1114	-1.3	2.2	hypothetical protein
TM1116	-1.3	5.1	hypothetical protein
TM1117	-1.2	4.3	general secretion pathway protein D, putative
TM1118	-1.3	6.3	hypothetical protein
TM1120	-1.1	1.5	glycerol-3-phosphate ABC transporter, periplasmic glycerol-3-phosphate-binding protein
TM1127	-2.3	3.2	hypothetical protein
TM1134	-1.1	3	hypothetical protein
TM1135	-2.1	8.1	branched chain amino acid ABC transporter, periplasmic amino acid-binding protein
TM1141	-1.3	7.6	cytochrome C-type biogenesis protein, putative
TM1143	-1.1	1.6	methyl-accepting chemotaxis protein
TM1165	-1.3	1.7	2-oxoacid ferredoxin oxidoreductase, beta subunit
TM1171	-1.4	5.3	transcriptional regulator, crp family
TM1172	-1.3	6	prismane protein
TM1177	-1.1	1.9	conserved hypothetical protein
TM1192	-1.3	5.3	alpha-galactosidase
TM1195	-1.6	2.7	beta-galactosidase
TM1197	-1.1	3.9	oligopeptide ABC transporter, permease protein
TM1198	-1.9	3.3	oligopeptide ABC transporter, permease protein
TM1201	-2.2	7.5	arabinogalactan endo-1,4-beta-galactosidase, putative
TM1203	-1.6	3.3	maltose ABC transporter, permease protein
TM1209	-1.2	2.3	conserved hypothetical protein
TM1214	-1.4	4.6	NADH dehydrogenase, putative
TM1215	-1.8	5.3	NADH dehydrogenase, 30 kDa subunit, putative
TM1216	-1.7	6.5	NADH dehydrogenase, 49 kDa subunit, putative
TM1224	-1.7	5.8	transcriptional regulator, XylR-related
TM1226	-1	9.7	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein, putative
TM1227	-2.1	10.6	endo-1,4-beta-mannosidase
TM1228	-1.9	8.3	transcriptional regulator, RpiR family
TM1231	-1	3.5	alpha-mannosidase-related protein
TM1232	-1.3	1.6	sugar ABC transporter, ATP-binding protein

TM1233	-1	1.6	sugar ABC transporter, permease protein, putative
TM1234	-1.2	2.8	sugar ABC transporter, permease protein
TM1235	-1.4	11.3	conserved hypothetical protein
TM1237	-1.2	3.4	conserved hypothetical protein
TM1254	-1	0.9	beta-phosphoglucomutase, putative
TM1271	-1.2	1.7	type IV pilin-related protein
TM1281	-1.4	1.6	6-phospho-beta-glucosidase
TM1291	-1	0.9	iron-sulfur cluster-binding protein
TM1310	-1.7	2.9	ABC transporter, ATP-binding protein
TM1365	-1.3	3.7	flagellar basal-body rod protein FlgC
TM1369	-1.6	2.1	conserved hypothetical protein
TM1372	-1.3	0.8	nitrogen fixation protein NifU-related protein
TM1374	-1.7	5.7	phosphoglycerate mutase
TM1375	-2.7	9.7	spermidine/putrescine ABC transporter, periplasmic spermidine/putrescine-binding protein
TM1400	-1.8	8.1	aspartate aminotransferase, putative
TM1411	-2.3	3.8	helicase-related protein
TM1415	-1.4	7.7	inositol monophosphatase family protein, putative
TM1427	-1	1	conserved hypothetical protein
TM1434	-1.5	2.2	hypothetical protein
TM1451	-2.3	10	RNA polymerase sigma-A factor
TM1458	-1.2	2.6	ribosomal protein L21
TM1485	-1.7	2.6	ribosomal protein L6
TM1525	-1.2	5.1	endoglucanase
TM1529	-1.5	4	hypothetical protein
TM1530	-1.5	3.5	electron transfer flavoprotein, beta subunit
TM1532	-1.3	5	fixC protein
TM1585	-1	2.7	glycerate kinase, putative
TM1598	-1.5	2.8	RNA polymerase sigma-E factor
TM1621	-1.1	2.5	hypothetical protein
TM1624	-1.3	2.6	beta-mannosidase, putative
TM1643	-1.1	0.8	conserved hypothetical protein
TM1667	-1	1.7	xylose isomerase
TM1706	-1.2	0.5	transcription elongation factor, greA/greB family
TM1721	-1	2.7	conserved hypothetical protein
TM1724	-1.5	1.2	3-oxoacyl-(acyl carrier protein) reductase
TM1731	-1.1	2	conserved hypothetical protein
TM1743	-1.3	7.6	oxidoreductase, aldo/keto reductase family
TM1746	-1.8	2.7	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM1747	-1	2.1	oligopeptide ABC transporter, permease protein
TM1749	-1.8	4.6	oligopeptide ABC transporter, ATP-binding protein
TM1752	-1	0.8	endoglucanase
TM1754	-1.6	3.7	butyrate kinase, putative
TM1755	-2.2	6.5	phosphate butyryltransferase
TM1756	-2	2.7	branched-chain-fatty-acid kinase, putative
TM1760	-1.2	1.4	
TM1777	-1.7	2.3	N utilization substance protein A
TM1778	-1.4	1.7	conserved hypothetical protein

TM1779	-1.2	1.3	hypothetical protein
TM1792	-1.1	1.7	conserved hypothetical protein
TM1801	-1	2	hypothetical protein
TM1805	-1.5	2.7	hypothetical protein
TM1819	-1.1	3	chromate transport protein, putative
TM1835	-1.1	0.4	cyclomaltodextrinase, putative
TM1840	-1.3	1.7	alpha-amylase
TM1844	-1	1.4	hypothetical protein
TM1853	-1.4	1	sugar ABC transporter, permease protein
TM1855	-1.1	1.9	sugar ABC transporter, periplasmic sugar-binding protein, putative
TM1866	-1.6	3.3	membrane bound protein LytR, putative
TMrrnaA16	-1.3	3.4	

TABLE 2.4. Differentially expressed ORFs for *T. maritima* batch challenge RM30 vs. WT30			
97 ORFs up-regulated in RM + 151 ORFs up-regulated in WT = 227 Total ORFs			
Gene	**Log(2) fold change RM30 vs. WT30**	**-log_{10}pval**	**Annotation**
TM0035	1	1.7	hypothetical protein
TM0036	1.7	6	conserved hypothetical protein
TM0038	1	6.6	6-pyruvoyl tetrahydrobiopterin synthase, putative
TM0039	1.4	7.7	conserved hypothetical protein
TM0040	1	5.2	dihydropteroate synthase
TM0092	1.3	10.6	hypothetical protein
TM0117	1.3	5.9	conserved hypothetical protein
TM0119	1.5	7.2	acetamidase, putative
TM0120	2.1	14.9	oxidoreductase, putative
TM0137	1.9	12.3	tryptophan synthase, alpha subunit
TM0147	1	10.2	conserved hypothetical protein
TM0159	1.1	5.9	ham1 protein
TM0164	1	3.2	conserved hypothetical protein
TM0169	1.4	6	conserved hypothetical protein
TM0172	1	1.7	adenosylhomocysteinase
TM0176	1.2	4.2	conserved hypothetical protein
TM0202	1.1	2.4	hypothetical protein
TM0203	1.1	2.8	ABC transporter, permease protein, cysTW family
TM0205	1.2	2.7	ATP-dependent DNA helicase
TM0206	1.3	2.6	hypoxanthine phosphoribosyltransferase
TM0207	1.6	7.8	conserved hypothetical protein
TM0213	1.3	1.3	glycine dehydrogenase (decarboxylating) subunit 1
TM0216	1.2	4	glycyl-tRNA synthetase, alpha subunit
TM0260	1.3	4.4	conserved hypothetical protein
TM0264	1.4	6.1	16S pseudouridylate synthase
TM0270	1	6.8	conserved hypothetical protein
TM0279	1.1	9	sugar ABC transporter, permease protein
TM0291	1.2	1.3	3-isopropylmalate dehydratase, large subunit, putative
TM0292	1.2	3.1	3-isopropylmalate dehydratase, small subunit, putative
TM0311	1.2	5.6	hypothetical protein
TM0312	2.3	15.4	conserved hypothetical protein
TM0313	1.5	17.8	K+ channel, beta subunit
TM0314	1	11.6	hypothetical protein
TM0318	1.1	4.1	ubiquinone/menaquinone biosynthesis-related protein
TM0321	1	6.7	hypothetical protein
TM0328	1.2	9.9	m4C-methyltransferase
TM0331	1.3	7.9	orotate phosphoribosyltransferase
TM0334	1.4	4.8	dihydroorotate dehydrogenase electron transfer protein
TM0382	1.6	9.4	repair endonuclease, putative
TM0384	1.5	5.2	anaerobic ribonucleoside-triphosphate reductase-related protein
TM0385	1.4	7.7	conserved hypothetical protein

TM0388	1.1	13.8	conserved hypothetical protein
TM0393	1.6	5.2	transcriptional regulator, XylR-related
TM0418	1.1	3.7	sugar ABC transporter, periplasmic sugar-binding protein, putative
TM0453	1	1.7	N utilization substance protein G
TM0455	1.1	4	ribosomal protein L1
TM0462	1.4	6.9	conserved hypothetical protein
TM0464	1.3	4.6	chemotactic methyltransferase
TM0465	1.2	3	hypothetical protein
TM0471	1.2	10.4	hypothetical protein
TM0488	1.1	4.4	hemK protein
TM0493	1.2	7.7	conserved hypothetical protein
TM0494	1	6.1	conserved hypothetical protein
TM0524	1.2	8.7	conserved hypothetical protein
TM0549	1.7	6.4	acetolactate synthase, small subunit
TM0589	1.1	6.7	hypothetical protein
TM0625	1	5.6	hypothetical protein
TM0626	1	7.7	hypothetical protein
TM0641	1.3	9.8	hypothetical protein
TM0642	1.1	4.8	hypothetical protein
TM0687	1.3	4.3	conserved hypothetical protein
TM0691	1.1	6	conserved hypothetical protein
TM0692	1	2.3	holo-(acyl carrier protein) synthase
TM0721	1.2	7.8	uracil phosphoribosyltransferase
TM0724	1.1	3.8	conserved hypothetical protein
TM0731	1	4.2	conserved hypothetical protein
TM0732	1.2	5.7	hypothetical protein
TM0739	1	5.5	hypothetical protein
TM0743	1.1	6.2	hypothetical protein
TM0767	1.4	10.2	maltodextrin glycosyltransferase
TM0768	1.4	10.9	conserved hypothetical protein
TM0801	1.3	8.3	(3R)-hydroxymyristoyl-(acyl carrier protein) dehydratase
TM0804	1.3	8.8	conserved hypothetical protein
TM0846	1.2	9	cytidine/deoxycytidine deaminase
TM0858	1.1	6.4	hypothetical protein
TM0860	1.1	2.8	protein-export membrane protein SecD, putative
TM0861	1	6.9	protein-export membrane protein SecF, putative
TM0867	1.2	9.1	hypothetical protein
TM0896	1	2.6	galactose-1-phosphate uridylyltransferase, putative
TM0899	1.7	6.5	hypothetical protein
TM0903	1.1	4.6	chemotaxis methylation protein
TM0906	1.1	4.8	conserved hypothetical protein
TM0911	1.2	7.6	translation initiation factor, aIF-2B alpha subunit-related
TM0913	1.2	7.6	mazG protein
TM0965	1	12.1	phosphoribosylaminoimidazole carboxylase-related protein
TM1003	1.3	3.7	transposase-related protein
TM1080	1.1	2.2	sugar-phosphate isomerase
TM1089	1	4.7	TRK system potassium uptake protein TrkH
TM1092	1	5.5	hypothetical protein

TM1123	1.3	7.2	flagellar hook-associated protein 2, putative
TM1125	1	8.3	hypothetical protein
TM1157	1.1	4.7	hypothetical protein
TM1167	1.4	12.7	hypothetical protein
TM1168	1.8	10.7	
TM1170	1.2	8.8	ABC transporter, periplasmic substrate-binding protein/conserved hypothetical protein
TM1246	1	1.8	phosphoribosylformylglycinamidine synthase II
TM1248	1	3.1	phosphoribosylglycinamide formyltransferase
TM1252	1.1	4.9	hypothetical protein
TM1261	1.4	11.2	phosphate ABC transporter, ATP-binding protein
TM1262	1.1	9.1	phosphate ABC transporter, permease protein
TM1264	1	3.9	phosphate ABC transporter, periplasmic phosphate-binding protein
TM1268	1.1	4.8	hypothetical protein
TM1274	1.5	8.1	hypothetical protein
TM1283	1.1	4.1	conserved hypothetical protein
TM1287	1.3	10.4	conserved hypothetical protein
TM1292	1.4	8.5	iron-sulfur cluster-binding protein, putative
TM1297	1	3.9	oxidoreductase, putative
TM1347	1.4	2.8	inosine-5'-monophosphate dehydrogenase
TM1369	1.4	7.1	conserved hypothetical protein
TM1380	1.1	2.9	conserved hypothetical protein
TM1381	1.2	5.1	hypothetical protein
TM1384	1	4.8	adenine phosphoribosyltransferase
TM1389	1	6.5	ubiquinone/menaquinone biosynthesis methyltransferase-related protein
TM1398	1	5.5	conserved hypothetical protein
TM1439	1.1	4.7	hypothetical protein
TM1443	1	2	cytidylate kinase
TM1460	1.4	6.8	jag protein, putative
TM1466	1	3.1	conserved hypothetical protein
TM1479	1.4	9.2	adenylate kinase
TM1500	1	2.1	ribosomal protein L3
TM1501	1.2	7.6	ribosomal protein S10
TM1558	1.1	6	conserved hypothetical protein
TM1586	1	7.4	hypothetical protein
TM1596	1.1	6.8	purine nucleoside phosphorylase
TM1607	1.2	3.8	conserved hypothetical protein
TM1611	1	1.3	ATP synthase F1, subunit gamma
TM1612	1.1	7.6	ATP synthase F1, subunit alpha
TM1614	1.1	10.7	ATP synthase F0, subunit b
TM1622	1.2	9.8	hypothetical protein
TM1626	1.1	3.7	peptidyl-tRNA hydrolase
TM1632	1.2	10.3	conserved hypothetical protein
TM1647	1.2	6.5	hypothetical protein
TM1679	1	7.7	conserved hypothetical protein
TM1685	1.6	16	conserved hypothetical protein
TM1732	1.1	7.9	conserved hypothetical protein

TM1733	1	3.5	conserved hypothetical protein
TM1761	1	6.3	excinuclease ABC, subunit B
TM1787	1.2	6.3	conserved hypothetical protein
TM1804	1	5.3	hypothetical protein
TM1806	1.2	6.3	hypothetical protein
TM1808	1	14	conserved hypothetical protein
TM1810	1.2	9.9	hypothetical protein
TM1813	1	6.5	hypothetical protein
TM1814	1.6	4.3	conserved hypothetical protein
TM1815	1	6.6	ferredoxin
TM1816	1.3	11.1	conserved hypothetical protein
TM1836	1	9	maltose ABC transporter, permease protein
TM1839	2.1	25.9	maltose ABC transporter, periplasmic maltose-binding protein
TM1842	1	6.1	hypothetical protein
TM1864	1.3	7.7	hypothetical protein
TM1874	1.6	11.1	cold shock protein
TM_tRNA-Cys-1	-1.1	11.2	
TM_tRNA-Leu-1	-1	5.6	
TM_tRNA-Leu-2	-1.1	4.7	
TM_tRNA-Met-3	-1	6.4	
TM0002	-1.3	11	hypothetical protein
TM0017	-1.3	3.2	pyruvate ferredoxin oxidoreductase, alpha subunit
TM0050	-1.4	3	iron(II) transport protein A
TM0058	-1.1	2.5	oligopeptide ABC transporter, ATP-binding protein
TM0095	-1	3.3	conserved hypothetical protein
TM0179	-1.1	10	hypothetical protein
TM0198	-2.6	10.6	ATP-dependent Clp protease, ATPase subunit
TM0209	-1.2	2.5	6-phosphofructokinase
TM0224	-1.3	3.4	biotin--(acetyl-CoA carboxylase) synthetase
TM0266	-1.1	2.5	DNA-binding protein, HU
TM0295	-1.1	3.3	transaldolase-related protein
TM0338	-1.3	6.7	hypothetical protein
TM0355	-1.2	4.5	hypothetical protein
TM0359	-1.4	9.8	conserved hypothetical protein
TM0365	-1	3.9	aminopeptidase, putative
TM0373	-1	6.2	dnaK protein
TM0374	-2.1	12.1	heat shock protein, class I
TM0423	-1.8	9.8	glycerol dehydrogenase
TM0429	-1.7	3.3	methyl-accepting chemotaxis protein
TM0435	-1.3	9.8	acetyl xylan esterase-related protein
TM0436	-1.5	13.9	alcohol dehydrogenase, zinc-containing
TM0437	-1.1	1.8	exo-poly-alpha-D-galacturonosidase, putative
TM0438	-1	6.7	6-phosphogluconate dehydrogenase, decarboxylating
TM0440	-1	6.7	hypothetical protein
TM0442	-1	3.6	conserved hypothetical protein

TM0445	-1.3	6.4	conserved hypothetical protein
TM0505	-1.8	9.5	groES protein
TM0506	-3	26.3	groEL protein
TM0558	-1.7	7	carbamoyl-phosphate synthetase, small subunit
TM0563	-1.1	10.9	conserved hypothetical protein
TM0568	-1	5.8	hypothetical protein
TM0570	-1.1	8.9	cell division protein FtsY
TM0571	-2.3	12.4	heat shock serine protease, periplasmic
TM0580	-1.2	5.4	cell division protein FtsH
TM0605	-1.2	9	ribosomal protein S18
TM0606	-1.6	15.3	hypothetical protein
TM0654	-3.5	15.1	spermidine synthase
TM0655	-2.4	26.7	conserved hypothetical protein
TM0656	-1.9	11.8	conserved hypothetical protein
TM0658	-1.4	11.8	neelaredoxin
TM0660	-1	3.2	conserved hypothetical protein
TM0677	-1.1	9.9	motility protein B
TM0747	-1.1	13.4	carboxyl-terminal protease
TM0755	-1	8.8	conserved hypothetical protein
TM0757	-1.1	8.1	hypothetical protein
TM0758	-3.2	36.3	flagellin
TM0762	-1.1	3.8	ribosomal protein S2
TM0771	-1.1	5.3	DNA polymerase III, gamma subunit-related protein
TM0807	-1	12.2	alkyl hydroperoxide reductase, putative
TM0818	-1.3	8.6	lipopolysaccharide biosynthesis protein, putative
TM0823	-1.4	9.7	transcriptional regulator, TetR family
TM0824	-1.3	8	astB/chuR-related protein
TM0916	-1.1	7.9	conserved hypothetical protein
TM0943	-2.3	20.3	glutamine synthetase
TM0980	-1	2.1	hypothetical protein
TM0982	-1.3	6.8	conserved hypothetical protein
TM0989	-1	11.6	conserved hypothetical protein
TM1045	-1	1.9	conserved hypothetical protein
TM1068	-1.2	15.4	alpha-glucosidase, putative
TM1135	-1.2	12.4	branched chain amino acid ABC transporter, periplasmic amino acid-binding protein
TM1182	-1.1	10	chromosome segregation SMC protein, putative
TM1196	-1.1	7.3	oligopeptide ABC transporter, ATP-binding protein
TM1216	-1	9	NADH dehydrogenase, 49 kDa subunit, putative
TM1227	-1	7.9	endo-1,4-beta-mannosidase
TM1229	-1	2.2	conserved hypothetical protein
TM1237	-1	7.8	conserved hypothetical protein
TM1242	-1.1	6.3	hypothetical protein
TM1254	-1	4	beta-phosphoglucomutase, putative
TM1275	-1	8	hypothetical protein
TM1326	-1	3	conserved hypothetical protein
TM1344	-1	4.3	ribosomal protein S15
TM1364	-1.3	3.3	flagellar basal-body rod protein FlgB
TM1366	-1.1	3.4	flagellar hook-basal body complex protein FliE

TM1393	-1.1	8	conserved hypothetical protein
TM1400	-1.3	14.2	aspartate aminotransferase, putative
TM1456	-1.2	3.4	ribosomal protein L27
TM1457	-1	1.3	hypothetical protein
TM1458	-1.8	10.7	ribosomal protein L21
TM1509	-1.1	6.1	conserved hypothetical protein
TM1511	-1.3	5.1	conserved hypothetical protein
TM1565	-1.1	10.3	signal recognition particle protein
TM1598	-1.6	12.7	RNA polymerase sigma-E factor
TM1655	-1.1	1.5	response regulator DrrA
TM1665	-1.3	8.6	hypothetical protein
TM1707	-1.3	4.7	conserved hypothetical protein
TM1719	-1.4	11.6	DNA mismatch repair protein
TM1720	-1.6	10	conserved hypothetical protein
TM1755	-1.1	6.7	phosphate butyryltransferase
TM1756	-1.5	7.7	branched-chain-fatty-acid kinase, putative
TM1760	-1.4	8.4	
TM1777	-2	9.3	N utilization substance protein A
TM1820	-1	3.5	GMP synthase
TM1845	-1	8.2	pullulanase

TABLE 2.5. Differentially expressed ORFs for *T. maritima* batch challenge RM00 vs. RM05			
32 ORFs up-regulated in RM at 0 + 28 ORFs up-regulated in RM at 5 min = 60 Total ORFs			
Gene	**Log (2) fold change RM00 vs. RM05**	**-$\log_{10}$pval**	**Annotation**
TM0060	1	6.3	oligopeptide ABC transporter, permease protein
TM0061	1.1	5.4	endo-1,4-beta-xylanase A
TM0063	1	5	hypothetical protein
TM0064	1	12.8	uronate isomerase, putative
TM0067	1.1	7.3	2-keto-3-deoxygluconate kinase
TM0069	1.1	5.9	D-mannonate hydrolase
TM0086	1.2	10.5	virulence factor MviN-related protein
TM0275	1.1	8.8	transcriptional regulator, GntR family
TM0281	1.1	9.9	alpha-L-arabinofuranosidase
TM0304	1.1	7.8	oligopeptide ABC transporter, ATP-binding protein
TM0305	1.2	6	endoglucanase, putative
TM0318	1	3.6	ubiquinone/menaquinone biosynthesis-related protein
TM0334	1.2	3.6	dihydroorotate dehydrogenase electron transfer protein
TM0344	1	4.6	prephenate dehydrogenase
TM0432	1	6.8	sugar ABC transporter, periplasmic sugar-binding protein, putative
TM1017	1	7.1	conserved hypothetical protein
TM1201	1	8.5	arabinogalactan endo-1,4-beta-galactosidase, putative
TM1218	1.2	11.7	transcriptional regulator, LacI family
TM1223	1.2	8.5	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM1422	1	6.7	rnfB-related protein
TM1755	1.1	7.7	phosphate butyryltransferase
TM1799	1	10.6	conserved hypothetical protein
TM1801	1	7.4	hypothetical protein
TM1806	1.1	5.6	hypothetical protein
TM1807	1.3	18	conserved hypothetical protein
TM1808	1	15.7	conserved hypothetical protein
TM1810	1	8.5	hypothetical protein
TM1811	1.1	11	conserved hypothetical protein
TM_rnpB	-1.3	12.6	
TM_TMrrnaA5S	-1	2.3	
TM0009	-1	1.8	hypothetical protein
TM0179	-1.1	10.2	hypothetical protein
TM0192	-1.3	9.9	spoVS-related protein
TM0255	-1	8	ribosomal protein L28
TM0266	-1	2.6	DNA-binding protein, HU
TM0374	-1.2	5.4	heat shock protein, class I
TM0456	-1.1	1.5	ribosomal protein L10
TM0504	-1.2	12.7	hypothetical protein
TM0505	-1.3	6.6	groES protein
TM0506	-1.1	8.7	groEL protein

TM0575	-1	2.2	crossover junction endodeoxyribonuclease
TM0576	-1.2	11.9	DNA polymerase III, alpha subunit
TM0604	-1.1	6	single stranded DNA-binding protein, putative
TM0605	-1.3	9.5	ribosomal protein S18
TM0762	-1.1	4.4	ribosomal protein S2
TM0980	-1.1	2.9	hypothetical protein
TM1241	-1.2	10.2	hypothetical protein
TM1497	-1.1	3.3	ribosomal protein L2
TM1498	-1	4	ribosomal protein L23
TM1505	-1	1.6	ribosomal protein S12
TM1565	-1.5	15.9	signal recognition particle protein
TM1568	-1	3.4	16S rRNA processing protein, putative
TM1569	-1	1.5	tRNA guanine-N1 methyltransferase
TM1570	-1	8.5	conserved hypothetical protein
TM1571	-1.2	2.1	ribosomal protein L19
TM1591	-1.2	5.8	ribosomal protein L35
TM1592	-1.3	3.2	ribosomal protein L20
TM1593	-1.3	3.7	conserved hypothetical protein
TM1685	-1.3	14.6	conserved hypothetical protein
TM1850	-1	1.1	hypothetical protein

TABLE 2.6. Differentially expressed ORFs for *T. maritima* batch challenge RM05 vs. RM30			
241 ORFs up-regulated in RM at 5 + 279 ORFs up-regulated in RM at 30 min = 520 Total ORFs			
Gene	**Log(2) fold change RM05 vs. RM30**	**-log_{10}pval**	**Annotation**
TM_tRNA-Leu-2	1	0.4	
TM0010	1.5	1.5	NADP-reducing hydrogenase, subunit C
TM0016	1.7	1.4	pyruvate ferredoxin oxidoreductase, delta subunit
TM0017	2.4	0.9	pyruvate ferredoxin oxidoreductase, alpha subunit
TM0018	1.9	11.4	pyruvate ferredoxin oxidoreductase, beta subunit
TM0031	1	0.3	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0035	1.6	1.9	hypothetical protein
TM0042	1.1	2.4	aminopeptidase P, putative
TM0050	2.5	0.8	iron(II) transport protein A
TM0080	1.2	2	iron(III) ABC transporter, periplasmic-binding protein, putative
TM0087	1.3	0.7	conserved hypothetical protein
TM0088	1.2	0.5	comE protein, putative
TM0089	1.3	0.7	hypothetical protein
TM0093	1.9	0.8	hypothetical protein
TM0097	1.2	0.4	conserved hypothetical protein
TM0098	1.6	0.5	conserved hypothetical protein
TM0099	1	0.4	hypothetical protein
TM0100	1.6	0.6	DNA ligase
TM0108	1.9	0.5	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
TM0118	1.7	5.3	ribonucleotide reductase, B12-dependent
TM0126	1	0.3	response regulator
TM0128	1	0.6	oxaloacetate decarboxylase, alpha subunit
TM0138	1.2	1.3	tryptophan synthase, beta subunit
TM0140	1.6	0.8	indole-3-glycerol phosphate synthase
TM0141	1.3	4.7	anthranilate synthase component II
TM0148	1.4	3	glucosamine--fructose-6-phosphate aminotransferase, isomerizing
TM0149	1.9	6.4	fatty acid/phospholipid synthesis protein
TM0166	1	1.1	folylpolyglutamate synthase/dihydrofolate synthase
TM0168	1.1	1.1	leucyl-tRNA synthetase
TM0181	1	1.6	hypothetical protein
TM0188	1.7	4.5	conserved hypothetical protein
TM0189	1.2	0.6	iron(III) ABC transporter, periplasmic iron-binding protein, putative
TM0190	1.2	0.4	iron(III) ABC transporter, permease protein, putative
TM0191	1.8	0.7	iron(III) ABC transporter, ATP-binding protein, putative
TM0196	1.5	0.8	conserved hypothetical protein
TM0198	1.1	0.6	ATP-dependent Clp protease, ATPase subunit
TM0204	1.4	0.7	ABC transporter, ATP-binding protein
TM0209	1.3	0.4	6-phosphofructokinase

TM0212	1.2	0.6	glycine cleavage system H protein
TM0214	1	1.1	glycine dehydrogenase (decarboxylating) subunit 2
TM0219	1	1	flagellar export/assembly protein
TM0220	1.5	4.5	flagellar motor switch protein FliG
TM0221	1.1	1.8	flagellar M-ring protein
TM0224	1.5	0.4	biotin--(acetyl-CoA carboxylase) synthetase
TM0225	1.1	0.5	1-aminocyclopropane-1-carboxylate deaminase, putative
TM0227	1.1	0.4	
TM0231	1.2	2.3	UDP-N-acetylmuramate--alanine ligase
TM0236	1.3	0.2	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate--D-alanyl-D-alanyl ligase
TM0239	1.2	0.8	glucose-1-phosphate adenylyltransferase
TM0240	1.3	0.5	glucose-1-phosphate adenylyltransferase
TM0253	1	0.7	conserved hypothetical protein
TM0255	1.2	1.5	ribosomal protein L28
TM0257	1.6	1.3	
TM0266	1	0.4	DNA-binding protein, HU
TM0290	2.3	2.1	citrate synthase
TM0295	1.6	0.8	transaldolase-related protein
TM0296	1.5	0.6	fructokinase
TM0313	1.3	2.6	K+ channel, beta subunit
TM0338	1	0.6	hypothetical protein
TM0355	1.7	1.1	hypothetical protein
TM0372	1.8	0.5	cation efflux system protein, putative
TM0374	1.3	0.6	heat shock protein, class I
TM0380	2.2	0.7	
TM0384	1.9	0.7	anaerobic ribonucleoside-triphosphate reductase-related protein
TM0386	1	1.3	bacterioferritin comigratory protein/NADH dehydrogenase
TM0392	1.4	0.4	conserved hypothetical protein
TM0397	1.5	4.1	glutamate synthase, alpha subunit
TM0398	1.3	1.9	conserved hypothetical protein
TM0406	1.1	0.9	keto/oxoacid ferredoxin oxidoreductase, gamma subunit, putative
TM0423	1.4	1.6	glycerol dehydrogenase
TM0429	2.9	0.8	methyl-accepting chemotaxis protein
TM0452	1.1	1.3	preprotein translocase SecE subunit
TM0454	4.1	1.4	ribosomal protein L11
TM0456	2.6	0.6	ribosomal protein L10
TM0457	1.5	0.8	ribosomal protein L7/L12
TM0458	1.5	7.2	DNA-directed RNA polymerase, beta subunit
TM0463	1.2	0.5	lipoprotein signal peptidase
TM0466	2	3	conserved hypothetical protein
TM0473	1.7	3.1	conserved hypothetical protein
TM0474	1.3	2.2	conserved hypothetical protein
TM0475	1.3	0.5	pyrazinamidase/nicotinamidase-related protein
TM0476	1	1	hypothetical protein
TM0478	1.4	2	tyrosyl-tRNA synthetase
TM0479	1.4	0.7	hypothetical protein

TM0498	1.6	2.2	oligopeptide ABC transporter, ATP-binding protein
TM0499	1.5	0.7	hypothetical protein
TM0501	1.8	1.7	oligopeptide ABC transporter, ATP-binding protein
TM0502	1.7	0.7	oligopeptide ABC transporter, permease protein
TM0505	1.1	0.6	groES protein
TM0506	1.7	1.9	groEL protein
TM0514	2.3	0.9	prolyl-tRNA synthetase
TM0525	1.3	2.1	tRNA delta-2-isopentenylpyrophosphate transferase
TM0526	1.2	3.1	host factor I
TM0530	1.5	1.4	oligopeptide ABC transporter, ATP-binding protein
TM0536	1	0.6	hypothetical protein
TM0542	1.4	2.7	malate oxidoreductase
TM0544	1.7	0.6	ABC transporter, ATP-binding protein
TM0545	1.2	0.5	homoserine kinase, putative
TM0546	2.3	0.8	threonine synthase
TM0550	1.4	2.9	ketol-acid reductoisomerase
TM0551	1.1	0.6	dihydroxy-acid dehydratase
TM0552	1	1.9	2-isopropylmalate synthase, putative
TM0558	1.3	1.3	carbamoyl-phosphate synthetase, small subunit
TM0567	1.1	0.5	conserved hypothetical protein
TM0575	2.5	1	crossover junction endodeoxyribonuclease
TM0576	1.5	2	DNA polymerase III, alpha subunit
TM0580	1.2	1.7	cell division protein FtsH
TM0581	1.1	0.7	hypothetical protein
TM0585	1.1	0.7	lipopolysaccharide biosynthesis protein BplA
TM0593	1.4	1.7	amino acid ABC transporter, periplasmic amino acid-binding protein
TM0599	1.2	1.8	hypothetical protein
TM0610	1	2.8	lipopolysaccharide biosynthesis protein
TM0613	1.2	0.6	conserved hypothetical protein
TM0635	1.9	1.6	hypothetical protein
TM0636	1.6	1.1	hypothetical protein
TM0645	1.2	0.4	NH(3)-dependent NAD(+) synthetase, putative
TM0654	1.1	0.9	spermidine synthase
TM0656	1.6	1.2	conserved hypothetical protein
TM0658	1.2	1.1	neelaredoxin
TM0660	1.3	0.7	conserved hypothetical protein
TM0661	1.2	6.4	hypothetical protein
TM0689	1.7	1.2	phosphoglycerate kinase/triose-phosphate isomerase
TM0693	1.4	0.6	hypothetical protein
TM0695	1.4	1.5	ATP-dependent Clp protease, proteolytic subunit
TM0700	1.9	2.6	chemotaxis response regulator CheY
TM0701	2.1	3.7	purine-binding chemotaxis protein
TM0703	1	0.8	competence-damage inducible protein, putative
TM0713	1	0.5	hypothetical protein
TM0717	1.3	3.4	propionyl-CoA carboxylase, gamma subunit
TM0718	1.4	1.4	purine-binding chemotaxis protein
TM0720	1.8	0.6	serine hydroxymethyltransferase
TM0722	1	0.8	vacB protein

TM0747	1.2	1.9	carboxyl-terminal protease
TM0748	1.1	1.2	conserved hypothetical protein
TM0762	2.3	2.1	ribosomal protein S2
TM0772	1.3	0.8	conserved hypothetical protein
TM0775	1	2.2	translation initiation factor IF-2
TM0785	1.1	1.6	bacteriocin
TM0786	1.4	1.3	hypothetical protein
TM0820	1.1	1.2	NADH-dependent butanol dehydrogenase, putative
TM0824	1.2	1.1	astB/chuR-related protein
TM0828	1.1	1.9	sugar kinase, pfkB family
TM0831	1.5	0.6	branched-chain amino acid aminotransferase, putative
TM0833	1.4	1.6	DNA gyrase, subunit B
TM0854	1.1	0.6	hypothetical protein
TM0859	1.2	0.6	hypothetical protein
TM0862	1.5	1.7	glucose-1-phosphate thymidylyltransferase
TM0872	1.3	1.3	conserved hypothetical protein
TM0887	1.1	3	methylated-DNA-protein-cysteine methyltransferase
TM0938	1.3	0.5	conserved hypothetical protein
TM0948	1.1	0.6	hypothetical protein
TM0972	1.4	0.2	conserved hypothetical protein, GGDEF domain
TM0979	1	1.4	conserved hypothetical protein
TM0980	2.5	1.1	hypothetical protein
TM0982	1.6	2	conserved hypothetical protein
TM0983	1.2	1.1	conserved hypothetical protein
TM1014	1.1	1.8	conserved hypothetical protein
TM1026	1.3	2.3	transposase, putative
TM1045	1.2	0.4	conserved hypothetical protein
TM1128	1.4	0.4	ferritin
TM1138	1.1	0.5	branched chain amino acid ABC transporter, ATP-binding protein
TM1145	1	1.5	conserved hypothetical protein
TM1147	1.1	0.4	conserved hypothetical protein
TM1148	1.9	5	isocitrate dehydrogenase
TM1161	1.7	1.4	Mg2+ transporter MgtE, putative
TM1239	1.2	1.2	conserved hypothetical protein
TM1240	1	0.7	conserved hypothetical protein
TM1241	1.6	1.9	hypothetical protein
TM1259	1.2	0.9	phosphate regulon transcriptional regulatory protein PhoB
TM1267	2.3	1.3	thiH protein, putative
TM1269	1.9	0.9	biotin synthetase, putative
TM1270	1.1	2.3	cystathionine gamma-synthase
TM1272	1	1.3	glutamyl tRNA-Gln amidotransferase, subunit A
TM1273	1.8	3.5	glutamyl tRNA-Gln amidotransferase, subunit B
TM1284	1	1.6	oxidase-related protein
TM1286	2.1	5.5	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
TM1314	1	1.4	conserved hypothetical protein
TM1316	1	0.4	hypothetical protein
TM1345	2.3	4.1	polynucleotide phosphorylase

TM1358	1	0.3	conserved hypothetical protein
TM1362	2.1	0.7	motility protein PilT
TM1363	1.7	2.3	peptide chain release factor RF-1
TM1383	1.7	1.1	conserved hypothetical protein
TM1386	1.3	0.7	hypothetical protein
TM1400	1.1	1.9	aspartate aminotransferase, putative
TM1401	1.7	5.7	D-3-phosphoglycerate dehydrogenase
TM1417	1	0.6	ABC transporter, ATP-binding protein
TM1420	1.6	1	hypothetical protein
TM1438	1.4	2.9	
TM1449	1.5	0.7	conserved hypothetical protein
TM1452	1.5	3	DNA primase
TM1454	1.2	7.8	ribosomal protein L13
TM1456	2.5	1.3	ribosomal protein L27
TM1457	2.4	1.3	hypothetical protein
TM1458	1.7	2.2	ribosomal protein L21
TM1459	2.8	2.9	conserved hypothetical protein
TM1461	1.2	1.1	inner membrane protein, putative
TM1463	1.6	2.4	ribonuclease P protein component
TM1469	1	2.9	glucokinase
TM1470	1.4	4.8	transcription termination factor Rho
TM1471	1.4	0.5	ribosomal protein L17
TM1474	2.1	1	ribosomal protein S11
TM1475	3.2	2.8	ribosomal protein S13
TM1477	1.5	1	
TM1480	1.2	0.4	preprotein translocase SecY subunit
TM1482	2.7	1.2	ribosomal protein L30
TM1486	1.1	0.3	ribosomal protein S8
TM1487	1.4	0.9	ribosomal protein S14
TM1488	1.2	1.5	ribosomal protein L5
TM1490	1.6	0.6	ribosomal protein L14
TM1491	1.2	0.6	ribosomal protein S17
TM1492	1.6	1.3	ribosomal protein L29
TM1493	2.5	1.5	ribosomal protein L16
TM1494	2.2	1.1	ribosomal protein S3
TM1497	1	0.3	ribosomal protein L2
TM1498	1	0.4	ribosomal protein L23
TM1500	1.4	2.2	ribosomal protein L3
TM1501	1.1	0.8	ribosomal protein S10
TM1502	1.7	0.6	translation elongation factor Tu
TM1503	1.3	0.7	translation elongation factor G
TM1505	3.4	1	ribosomal protein S12
TM1506	2	0.7	conserved hypothetical protein
TM1507	1.2	1.7	phoH-related protein
TM1509	2.1	1.6	conserved hypothetical protein
TM1511	1.8	0.9	conserved hypothetical protein
TM1512	2.1	0.7	sun protein
TM1522	2.5	1	diaminopimelate epimerase
TM1546	1.5	0.4	single stranded DNA-specific exonuclease, putative

TM1565	1.6	3.4	signal recognition particle protein
TM1566	2.1	0.7	ribosomal protein S16
TM1571	3.2	1.2	ribosomal protein L19
TM1590	2.4	2.8	translation initiation factor IF-3
TM1591	2.6	2	ribosomal protein L35
TM1592	2.7	1.7	ribosomal protein L20
TM1595	1.7	1.7	conserved hypothetical protein
TM1597	1.1	1	hypothetical protein
TM1609	1.4	1.8	ATP synthase F1, subunit epsilon
TM1612	1.3	1.2	ATP synthase F1, subunit alpha
TM1615	2.7	0.8	ATP synthase F0, subunit c
TM1618	1.5	1	cheX protein
TM1627	1.9	1.6	general stress protein Ctc
TM1628	1.9	2.4	phosphoribosyl pyrophosphate synthetase
TM1630	1	0.5	hypothetical protein
TM1631	1.3	0.8	conserved hypothetical protein
TM1634	1.1	0.8	hypothetical protein
TM1646	1	0.8	conserved hypothetical protein
TM1649	1.2	0.5	conserved hypothetical protein
TM1655	1.6	0.3	response regulator DrrA
TM1657	1.3	1.5	ribosomal protein S20
TM1658	2.5	3.5	S-adenosylmethionine synthetase
TM1662	1.4	0.9	stationary phase survival protein
TM1663	1	1	ABC transporter, ATP-binding protein
TM1664	1	0.8	conserved hypothetical protein
TM1665	1.4	3.8	hypothetical protein
TM1684	1.5	0.4	ribosomal protein L31
TM1688	1.3	1.2	hypothetical protein
TM1691	1.9	0.8	conserved hypothetical protein
TM1697	1.1	0.8	conserved hypothetical protein
TM1704	1.8	0.6	hypothetical protein
TM1705	1.9	2.6	lysyl-tRNA synthetase
TM1707	1.5	0.8	conserved hypothetical protein
TM1715	1.2	1.7	conserved hypothetical protein
TM1763	1.5	0.8	translation elongation factor P
TM1765	1.4	1.2	N utilization substance protein B
TM1767	2.5	1.1	methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase
TM1769	1.2	0.6	exodeoxyribonuclease, small subunit
TM1774	1.2	0.9	phosphonopyruvate decarboxylase, putative
TM1782	1.2	1.6	N-acetyl-gamma-glutamyl-phosphate reductase
TM1785	1.2	3.4	acetylornithine aminotransferase
TM1796	1.1	0.5	conserved hypothetical protein
TM1809	1.6	0.8	conserved hypothetical protein
TM1812	1	0.8	hypothetical protein
TM1820	1.4	1.3	GMP synthase
TM1832	1.3	1.5	transposase
TM1858	1.6	3.1	recX protein, putative
TM1859	1.6	1.1	DNA repair protein

TM1870	1.4	1.2	septum site-determining protein MinD
TM1871	1.5	0.8	hypothetical protein
TM1877	1.3	0.5	conserved hypothetical protein
TM_tRNA-Gln-2	-1.5	2.9	
TM_tRNA-Glu-2	-1.4	1.4	
TM_tRNA-Gly-2	-1.6	8.8	
TM0004	-1.9	3.1	hypothetical protein
TM0028	-1	0.8	oligopeptide ABC transporter, ATP-binding protein
TM0036	-1.3	0.7	conserved hypothetical protein
TM0055	-1.4	4.5	alpha-glucuronidase
TM0059	-1.7	2.1	oligopeptide ABC transporter, permease protein
TM0062	-1.1	1.2	hypothetical protein
TM0066	-1.1	1.2	2-dehydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate aldolase
TM0070	-1.5	2.1	endo-1,4-beta-xylanase B
TM0071	-1.2	0.4	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0073	-1.4	1.4	oligopeptide ABC transporter, permease protein
TM0078	-1	0.9	iron(III) ABC transporter, ATP-binding protein
TM0082	-1.3	1	flagellar hook-associated protein 3
TM0083	-1.3	1.7	flagellar hook-associated protein 1
TM0086	-1.2	1.8	virulence factor MviN-related protein
TM0112	-1	0.5	sugar ABC transporter, permease protein
TM0114	-1.1	0.5	sugar ABC transporter, periplasmic sugar-binding protein
TM0117	-2.8	3.3	conserved hypothetical protein
TM0120	-2.2	2.6	oxidoreductase, putative
TM0131	-1.1	1	
TM0134	-1.1	1	thioredoxin reductase-related protein
TM0157	-1	1.8	actinorhodin polyketide dimerase-related protein
TM0185	-1.9	0.7	hypothetical protein
TM0187	-1	1.1	
TM0207	-2.5	2.2	conserved hypothetical protein
TM0211	-1.3	2.2	aminomethyltransferase
TM0213	-2	1.6	glycine dehydrogenase (decarboxylating) subunit 1
TM0229	-1	0.4	conserved hypothetical protein
TM0230	-1	1.2	conserved hypothetical protein
TM0241	-1.4	1.8	hypothetical protein
TM0256	-1.5	1.3	hypothetical protein
TM0260	-1.9	0.7	conserved hypothetical protein
TM0264	-2.6	2.1	16S pseudouridylate synthase
TM0269	-1.3	5.3	hypothetical protein
TM0270	-2.8	9.5	conserved hypothetical protein
TM0279	-1	0.8	sugar ABC transporter, permease protein
TM0281	-1	1.6	alpha-L-arabinofuranosidase
TM0291	-1.8	1.5	3-isopropylmalate dehydratase, large subunit, putative
TM0301	-1.2	0.8	oligopeptide ABC transporter, permease protein
TM0302	-1.6	5.6	oligopeptide ABC transporter, permease protein
TM0304	-1.2	1.7	oligopeptide ABC transporter, ATP-binding protein
TM0305	-1.1	0.7	endoglucanase, putative

TM0310	-1.2	0.6	beta-D-galactosidase
TM0311	-1.3	0.7	hypothetical protein
TM0317	-1.2	1.9	cation-transporting ATPase, P-type
TM0318	-1.1	0.6	ubiquinone/menaquinone biosynthesis-related protein
TM0320	-1.5	1.2	heavy metal binding protein
TM0321	-2.6	5.8	hypothetical protein
TM0322	-1.2	0.8	ABC transporter, periplasmic substrate-binding protein, putative
TM0323	-1.8	1.8	
TM0324	-2.3	1.1	conserved hypothetical protein
TM0329	-1.8	5	hypothetical protein
TM0333	-1.6	1.8	dihydroorotate dehydrogenase
TM0334	-4.4	2.7	dihydroorotate dehydrogenase electron transfer protein
TM0349	-1.1	0.9	3-dehydroquinase dehydratase
TM0361	-1	1.1	hypothetical protein
TM0376	-1.5	2	conserved hypothetical protein
TM0382	-2	1.5	repair endonuclease, putative
TM0383	-1.1	2.9	NADH oxidoreductase, putative
TM0405	-1.2	1	keto/oxoacid ferredoxin oxidoreductase, beta subunit, putative
TM0411	-1.1	1.4	transcriptional regulator, XylR-related
TM0418	-1.2	0.5	sugar ABC transporter, periplasmic sugar-binding protein, putative
TM0420	-1.1	0.6	sugar ABC transporter, permease protein
TM0431	-1	0.7	sugar ABC transporter, permease protein
TM0432	-1.5	2.1	sugar ABC transporter, periplasmic sugar-binding protein, putative
TM0441	-1.3	1.3	oxidoreductase, short chain dehydrogenase/reductase family
TM0442	-1	0.8	conserved hypothetical protein
TM0455	-1.4	1.2	ribosomal protein L1
TM0462	-2.3	2.7	conserved hypothetical protein
TM0465	-1.2	0.4	hypothetical protein
TM0485	-1.6	0.7	ABC transporter, permease protein, cysTW family
TM0493	-1.1	0.7	conserved hypothetical protein
TM0494	-1.1	0.8	conserved hypothetical protein
TM0504	-1	1.1	hypothetical protein
TM0517	-1.3	2.8	hypothetical protein
TM0535	-1	0.4	hypothetical protein
TM0549	-2.2	1.5	acetolactate synthase, small subunit
TM0561	-1.5	0.9	divalent cation transport-related protein
TM0562	-2.2	10.3	hypothetical protein
TM0589	-2.6	3.1	hypothetical protein
TM0625	-1.4	1	hypothetical protein
TM0640	-2.2	4.2	conserved hypothetical protein
TM0642	-2	2.5	hypothetical protein
TM0651	-2.1	2.9	conserved hypothetical protein
TM0665	-1.8	1.3	cysteine synthase
TM0666	-1.4	0.7	serine acetyltransferase
TM0681	-1.8	2.1	dehydrase-related protein

TM0692	-1.6	0.9	holo-(acyl carrier protein) synthase
TM0698	-1.1	0.4	flagellar biosynthesis protein FliP
TM0706	-1.3	1.9	hypothetical protein
TM0710	-1.2	0.8	transcriptional regulator, MarR family
TM0724	-1.2	0.6	conserved hypothetical protein
TM0725	-1.3	4.5	hypothetical protein
TM0731	-2.1	1.7	conserved hypothetical protein
TM0777	-1.1	0.8	transposase
TM0778	-2.3	3.1	hypothetical protein
TM0779	-1	0.4	conserved hypothetical protein
TM0784	-1.9	5.5	hypothetical protein
TM0789	-1.3	1	hypothetical protein
TM0795	-1.3	1	sugar kinase, pfkB family
TM0810	-1.4	5.6	sugar ABC transporter, periplasmic sugar-binding protein
TM0811	-1	0.9	sugar ABC transporter, permease protein
TM0813	-1.5	0.9	conserved hypothetical protein
TM0816	-1.1	1.4	transcriptional regulator, putative, Mar family
TM0826	-1	2.5	hypothetical protein
TM0840	-1.6	2.2	hypothetical protein
TM0851	-1.1	1.5	heat shock operon repressor HrcA
TM0858	-1	0.8	hypothetical protein
TM0860	-1.3	0.5	protein-export membrane protein SecD, putative
TM0865	-1.6	1.6	conserved hypothetical protein
TM0867	-1.1	1.2	hypothetical protein
TM0882	-1	0.6	O-acetylhomoserine sulfhydrylase
TM0888	-1.5	3.3	conserved hypothetical protein
TM0892	-1	0.6	hypothetical protein
TM0899	-3.6	2.3	hypothetical protein
TM0903	-2.1	2.7	chemotaxis methylation protein
TM0906	-2.2	2.8	conserved hypothetical protein
TM0911	-1.4	1.2	translation initiation factor, aIF-2B alpha subunit-related
TM0913	-1.4	1.3	mazG protein
TM0927	-1.3	2.6	ferredoxin
TM0934	-2.5	2.9	conserved hypothetical protein
TM0959	-1.2	1.7	ribose ABC transporter, membrane-associated protein
TM0967	-1.1	1.6	integrase-recombinase protein
TM0985	-1	1.7	hypothetical protein
TM0995	-1.1	0.4	hypothetical protein
TM0998	-1.2	3.7	heavy metal resistance transcriptional regulator
TM1001	-2	2	conserved hypothetical protein
TM1002	-1.4	1.3	conserved hypothetical protein
TM1009	-1.5	1.5	oxidoreductase, aldo/keto reductase family
TM1016	-2.8	2.9	hypothetical protein
TM1017	-1.2	1.3	conserved hypothetical protein
TM1027	-1.3	2.1	hypothetical protein
TM1029	-1.2	0.9	ABC transporter, permease protein, putative
TM1030	-1.3	1.5	transcriptional regulator, TetR family
TM1035	-2.5	1.5	phosphoribosyl-AMP cyclohydrolase / phosphoribosyl-ATP pyrophosphohydrolase

TM1083	-1.8	0.9	conserved hypothetical protein
TM1091	-2.2	2.7	hypothetical protein
TM1105	-1.4	1.1	NADH dehydrogenase, putative
TM1107	-1.3	0.5	conserved hypothetical protein
TM1108	-1.2	1.3	hypothetical protein
TM1120	-1.8	1.4	glycerol-3-phosphate ABC transporter, periplasmic glycerol-3-phosphate-binding protein
TM1121	-1.3	1.4	glycerol-3-phosphate ABC transporter, permease protein
TM1123	-2.4	3.1	flagellar hook-associated protein 2, putative
TM1127	-2.7	1.9	hypothetical protein
TM1129	-1.1	1.7	5-methylthioadenosine/S-adenosylhomocysteine nucleosidase
TM1141	-1.4	5.3	cytochrome C-type biogenesis protein, putative
TM1154	-1.1	0.7	oxidoreductase, sol/devB family
TM1157	-1.2	0.7	hypothetical protein
TM1165	-2.1	1.8	2-oxoacid ferredoxin oxidoreductase, beta subunit
TM1177	-2.1	2.7	conserved hypothetical protein
TM1185	-1	1.1	methylglyoxal synthase
TM1198	-1.3	0.9	oligopeptide ABC transporter, permease protein
TM1201	-1.2	1.5	arabinogalactan endo-1,4-beta-galactosidase, putative
TM1203	-1.5	1.4	maltose ABC transporter, permease protein
TM1205	-1.1	1.2	conserved hypothetical protein
TM1206	-1.7	2.2	conserved hypothetical protein
TM1207	-1.5	1.9	conserved hypothetical protein
TM1215	-2.6	4.5	NADH dehydrogenase, 30 kDa subunit, putative
TM1224	-1.5	2.2	transcriptional regulator, XylR-related
TM1227	-1.1	2.3	endo-1,4-beta-mannosidase
TM1228	-1.1	1.9	transcriptional regulator, RpiR family
TM1232	-1.2	0.7	sugar ABC transporter, ATP-binding protein
TM1237	-1	1.1	conserved hypothetical protein
TM1265	-1.5	2.5	conserved hypothetical protein
TM1279	-1.6	1.6	hypothetical protein
TM1281	-1.3	0.7	6-phospho-beta-glucosidase
TM1283	-1.3	0.6	conserved hypothetical protein
TM1285	-1	13.7	hypothetical protein
TM1289	-1.3	1.1	ferredoxin
TM1292	-1.3	0.8	iron-sulfur cluster-binding protein, putative
TM1310	-1.4	1	ABC transporter, ATP-binding protein
TM1330	-1	2.8	lacI family transcriptional regulator, putative
TM1336	-1	0.4	permease, putative
TM1343	-1.1	1.9	
TM1350	-1.1	0.6	lipase, putative
TM1365	-1.2	1.5	flagellar basal-body rod protein FlgC
TM1369	-3.6	3.6	conserved hypothetical protein
TM1372	-3	1.3	nitrogen fixation protein NifU-related protein
TM1374	-1.9	4.5	phosphoglycerate mutase
TM1375	-1.3	1.7	spermidine/putrescine ABC transporter, periplasmic spermidine/putrescine-binding protein
TM1388	-1.1	1	conserved hypothetical protein

TM1389	-1	0.9	ubiquinone/menaquinone biosynthesis methyltransferase-related protein
TM1411	-3.7	3.7	helicase-related protein
TM1415	-2.4	11.3	inositol monophosphatase family protein, putative
TM1427	-1.7	1	conserved hypothetical protein
TM1431	-1	5.6	glycerol uptake operon antiterminator
TM1434	-1.2	0.9	hypothetical protein
TM1444	-1.1	3.6	lytB protein
TM1451	-1.7	4.2	RNA polymerase sigma-A factor
TM1460	-2.2	2.8	jag protein, putative
TM1466	-1.3	0.9	conserved hypothetical protein
TM1468	-1.2	4.4	conserved hypothetical protein
TM1485	-2.3	2	ribosomal protein L6
TM1526	-1.5	0.7	hypothetical protein
TM1529	-2.7	4.7	hypothetical protein
TM1530	-1.9	2.8	electron transfer flavoprotein, beta subunit
TM1540	-1.8	1.9	flagellar L-ring protein
TM1542	-1.4	1.8	flagellar basal-body rod protein FlgG
TM1545	-1	2.2	conserved hypothetical protein
TM1559	-1.2	0.2	deoxyribose-phosphate aldolase
TM1621	-2.2	4.1	hypothetical protein
TM1626	-1.9	2.3	peptidyl-tRNA hydrolase
TM1643	-1.2	0.5	conserved hypothetical protein
TM1645	-1.2	0.7	nicotinate-nucleotide pyrophosphorylase
TM1667	-2.3	2.7	xylose isomerase
TM1676	-1.6	1	hypothetical protein
TM1679	-1.1	0.8	conserved hypothetical protein
TM1681	-1.2	1.1	hypothetical protein
TM1693	-1.4	0.6	1-acyl-sn-glycerol-3-phosphate acetyltransferase, putative
TM1716	-1.4	3.6	hypothetical protein
TM1724	-2.4	1.2	3-oxoacyl-(acyl carrier protein) reductase
TM1731	-2.5	3.6	conserved hypothetical protein
TM1741	-1	1.1	conserved hypothetical protein
TM1742	-1	0.4	nagD protein, putative
TM1743	-1.1	3.2	oxidoreductase, aldo/keto reductase family
TM1746	-1.6	1.1	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM1747	-1.2	1.3	oligopeptide ABC transporter, permease protein
TM1749	-1.5	1.7	oligopeptide ABC transporter, ATP-binding protein
TM1754	-1.3	1.3	butyrate kinase, putative
TM1755	-1	1.1	phosphate butyryltransferase
TM1761	-1.2	1.3	excinuclease ABC, subunit B
TM1775	-1.2	1.1	hypothetical protein
TM1779	-1.5	0.9	hypothetical protein
TM1787	-2.2	2.8	conserved hypothetical protein
TM1789	-1.1	1.7	hypothetical protein
TM1798	-1.2	0.8	conserved hypothetical protein
TM1800	-1.1	2.4	hypothetical protein
TM1804	-1	0.7	hypothetical protein

TM1805	-3	3.8	hypothetical protein
TM1808	-1.7	6.8	conserved hypothetical protein
TM1814	-2.9	2.4	conserved hypothetical protein
TM1824	-1.1	3.4	hypothetical protein
TM1833	-1	0.6	methyl-accepting chemotaxis-related protein
TM1835	-1.5	0.3	cyclomaltodextrinase, putative
TM1840	-1.9	1.4	alpha-amylase
TM1866	-1.1	0.9	membrane bound protein LytR, putative
TM1868	-1.3	2.2	conserved hypothetical protein
TM1874	-2.5	2.1	cold shock protein
TMrrnaA16	-1.3	1.6	

TABLE 2.7. Differentially expressed ORFs for *T. maritima* batch CAM challenge WT00 vs. WT05			
139 ORFs up-regulated in WT at 0 + 135 ORFs up-regulated in WT at 5 min = 274 Total ORF			
Gene	**Log(2) fold change WT00 vs. WT05**	**-log_{10}pval**	**Annotation**
TM0035	2.2	7.7	hypothetical protein
TM0036	1	2.9	conserved hypothetical protein
TM0039	1.2	6.8	conserved hypothetical protein
TM0042	1.2	16.5	aminopeptidase P, putative
TM0053	1	2.4	esterase, putative
TM0054	1.1	12.8	hypothetical protein
TM0089	1	3.2	hypothetical protein
TM0092	1.2	11	hypothetical protein
TM0093	1	2.5	hypothetical protein
TM0137	1.9	13.5	tryptophan synthase, alpha subunit
TM0146	1.2	10.1	ATP-dependent Clp protease, ATPase subunit clpX
TM0149	1.1	13.4	fatty acid/phospholipid synthesis protein
TM0172	1.2	2.4	adenosylhomocysteinase
TM0206	1.2	2.4	hypoxanthine phosphoribosyltransferase
TM0220	1	13	flagellar motor switch protein FliG
TM0274	1.4	7.7	acetate kinase
TM0275	1.2	9.9	transcriptional regulator, GntR family
TM0293	1	2.1	gamma-glutamyl phosphate reductase
TM0312	1.8	11.9	conserved hypothetical protein
TM0313	1.9	23.9	K+ channel, beta subunit
TM0318	1.1	4.4	ubiquinone/menaquinone biosynthesis-related protein
TM0331	1	5.8	orotate phosphoribosyltransferase
TM0386	1.3	13.6	bacterioferritin comigratory protein/NADH dehydrogenase
TM0393	1.7	7.8	transcriptional regulator, XylR-related
TM0394	1.9	5.5	conserved hypothetical protein
TM0395	1.9	14.3	NADH oxidase, putative
TM0396	1.7	19.1	iron-sulfur cluster-binding protein
TM0397	1.7	21.4	glutamate synthase, alpha subunit
TM0398	1.3	7.3	conserved hypothetical protein
TM0463	1.2	4.6	lipoprotein signal peptidase
TM0471	1.2	12	hypothetical protein
TM0488	1	4.4	hemK protein
TM0504	1.3	14.2	hypothetical protein
TM0524	1.3	9.5	conserved hypothetical protein
TM0542	1.1	11.7	malate oxidoreductase
TM0544	1.3	3.5	ABC transporter, ATP-binding protein
TM0545	2.2	7.6	homoserine kinase, putative
TM0546	1.3	3.2	threonine synthase
TM0548	1.3	7.4	acetolactate synthase, large subunit
TM0549	1.2	4.4	acetolactate synthase, small subunit
TM0550	1.5	15.6	ketol-acid reductoisomerase

TM0551	1.1	4.3	dihydroxy-acid dehydratase
TM0552	1	6.4	2-isopropylmalate synthase, putative
TM0553	1.2	9.8	2-isopropylmalate synthase
TM0554	1.3	11.4	3-isopropylmalate dehydratase, large subunit
TM0555	1.6	9.7	3-isopropylmalate dehydratase, small subunit
TM0621	1.1	8.7	
TM0630	1.3	16.7	nucleotide sugar epimerase, putative
TM0641	1.5	11.4	hypothetical protein
TM0664	1.2	8.1	conserved hypothetical protein
TM0665	2.2	14.3	cysteine synthase
TM0666	1.8	9.1	serine acetyltransferase
TM0687	1.5	6.1	conserved hypothetical protein
TM0712	1.3	7.1	conserved hypothetical protein
TM0713	1.3	5.1	hypothetical protein
TM0714	1.2	18.2	hypothetical protein
TM0720	1.5	3.6	serine hydroxymethyltransferase
TM0721	1.3	8.7	uracil phosphoribosyltransferase
TM0733	1	7.5	sigma-B regulator, putative
TM0753	1.3	11.2	ubiquinone/menaquinone biosynthesis methyltransferase, putative
TM0754	1.1	8.1	oxidoreductase
TM0804	1.8	13.7	conserved hypothetical protein
TM0831	1.3	4.2	branched-chain amino acid aminotransferase, putative
TM0837	1	15.2	general secretion pathway protein E
TM0859	1.2	6.1	hypothetical protein
TM0861	1.2	9.6	protein-export membrane protein SecF, putative
TM0862	1	7.1	glucose-1-phosphate thymidylyltransferase
TM0868	1.4	18.5	glutaredoxin-related protein
TM0881	1.8	19.1	homoserine O-succinyltransferase
TM0896	1.6	5.7	galactose-1-phosphate uridylyltransferase, putative
TM0965	2	24.7	phosphoribosylaminoimidazole carboxylase-related protein
TM0966	2.1	11.3	conserved hypothetical protein
TM1078	1	6.1	conserved hypothetical protein
TM1081	1.1	9.8	anti-sigma factor antagonist, putative
TM1111	1	7.5	hypothetical protein
TM1148	1.2	6	isocitrate dehydrogenase
TM1150	1.3	7.5	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM1151	1.4	10.8	oligopeptide ABC transporter, ATP-binding protein
TM1152	1.5	9.9	oligopeptide ABC transporter, ATP-binding protein
TM1153	1	3.3	oligopeptide ABC transporter, permease protein
TM1154	1.7	7.1	oxidoreductase, sol/devB family
TM1155	2	8.5	glucose-6-phosphate 1-dehydrogenase
TM1218	1.5	16.6	transcriptional regulator, LacI family
TM1219	1.6	16	oligopeptide ABC transporter, ATP-binding protein
TM1220	1.5	17.7	oligopeptide ABC transporter, ATP-binding protein
TM1221	1.2	4.3	oligopeptide ABC transporter, permease protein
TM1239	1.1	2.6	conserved hypothetical protein
TM1243	1.2	10.2	phosphoribosylaminoimidazole-succinocarboxamide

			synthase
TM1244	1.4	4.8	conserved hypothetical protein
TM1245	2.3	25.2	phosphoribosylformylglycinamidine synthase I
TM1246	1.7	5.4	phosphoribosylformylglycinamidine synthase II
TM1247	1.2	10.4	amidophosphoribosyltransferase
TM1248	1.7	8.1	phosphoribosylglycinamide formyltransferase
TM1249	1.5	15.7	phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
TM1251	1.1	9.8	phosphoribosylformylglycinamidine cyclo-ligase
TM1252	1.5	8.4	hypothetical protein
TM1267	2.6	12.4	thiH protein, putative
TM1268	1	5.1	hypothetical protein
TM1269	1.4	4.6	biotin synthetase, putative
TM1270	1	7.2	cystathionine gamma-synthase
TM1273	1.4	10.8	glutamyl tRNA-Gln amidotransferase, subunit B
TM1274	1.6	9.7	hypothetical protein
TM1286	1.7	16	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
TM1345	1.2	11.2	polynucleotide phosphorylase
TM1346	1.2	8.3	processing protease, putative
TM1347	1.6	3.6	inosine-5'-monophosphate dehydrogenase
TM1362	1.4	3.7	motility protein PilT
TM1369	1	4.9	conserved hypothetical protein
TM1370	2.8	24.6	hypothetical protein
TM1371	2.2	24.3	aminotransferase, class V
TM1380	1.1	3.5	conserved hypothetical protein
TM1381	1.5	7.9	hypothetical protein
TM1383	1.1	4.3	conserved hypothetical protein
TM1461	1.6	7.7	inner membrane protein, putative
TM1536	1.3	9.5	conserved hypothetical protein
TM1537	1	8.2	Mg-protoporphyrin IX monomethyl ester oxidative cyclase-related protein
TM1561	1.1	10.8	tRNA guanine transglycosylase
TM1589	1	3.1	clostripain-related protein
TM1595	1	6.5	conserved hypothetical protein
TM1607	1.1	3.2	conserved hypothetical protein
TM1610	1.8	18.7	ATP synthase F1, subunit beta
TM1611	2	5.6	ATP synthase F1, subunit gamma
TM1612	2	17.4	ATP synthase F1, subunit alpha
TM1613	1	14.5	ATP synthase F1, subunit delta
TM1651	1	12.7	translation elongation factor G
TM1697	1.1	7.2	conserved hypothetical protein
TM1704	1.2	3.1	hypothetical protein
TM1705	1	8.8	lysyl-tRNA synthetase
TM1806	1.4	7.9	hypothetical protein
TM1807	1.3	19	conserved hypothetical protein
TM1809	1.2	3.1	conserved hypothetical protein
TM1811	1.3	14.5	conserved hypothetical protein
TM1812	1.1	4.7	hypothetical protein

TM1813	1.1	8.1	hypothetical protein
TM1817	1	8.7	valyl-tRNA synthetase
TM0044	-1.7	6.7	hypothetical protein
TM0048	-1.1	10.2	transposase
TM0050	-1	2	iron(II) transport protein A
TM0059	-1	9	oligopeptide ABC transporter, permease protein
TM0079	-1	7.8	iron(III) ABC transporter, permease protein
TM0144	-1.6	8.3	conserved hypothetical protein
TM0150	-1.2	4.8	ribosomal protein L32
TM0152	-1	3.6	hypothetical protein
TM0160	-1.1	10.6	conserved hypothetical protein
TM0167	-1.2	8	phosphopentomutase
TM0179	-2	21.2	hypothetical protein
TM0192	-2	18	spoVS-related protein
TM0198	-1.1	3.7	ATP-dependent Clp protease, ATPase subunit
TM0209	-1	2	6-phosphofructokinase
TM0255	-1.4	12.4	ribosomal protein L28
TM0266	-2.8	11.9	DNA-binding protein, HU
TM0297	-1.2	7.2	oxidoreductase, short chain dehydrogenase/reductase family
TM0338	-1.8	11.2	hypothetical protein
TM0352	-1.1	9.2	ABC transporter, ATP-binding protein
TM0369	-2.8	22.3	conserved hypothetical protein
TM0370	-1.2	8.7	conserved hypothetical protein
TM0379	-1.2	7.8	NADH oxidase
TM0411	-1	8.6	transcriptional regulator, XylR-related
TM0431	-1.2	7.1	sugar ABC transporter, permease protein
TM0432	-1.1	8.2	sugar ABC transporter, periplasmic sugar-binding protein, putative
TM0433	-1.5	5.9	pectate lyase
TM0435	-1.4	11.5	acetyl xylan esterase-related protein
TM0436	-1.6	15.9	alcohol dehydrogenase, zinc-containing
TM0437	-1.1	1.8	exo-poly-alpha-D-galacturonosidase, putative
TM0438	-1.1	9.3	6-phosphogluconate dehydrogenase, decarboxylating
TM0439	-1	7.1	transcriptional regulator, GntR family
TM0441	-1.3	9.3	oxidoreductase, short chain dehydrogenase/reductase family
TM0442	-1.4	6.9	conserved hypothetical protein
TM0445	-1.1	6	conserved hypothetical protein
TM0451	-1.2	6.7	ribosomal protein L33
TM0455	-1.1	5	ribosomal protein L1
TM0473	-1.4	5	conserved hypothetical protein
TM0480	-1	4.1	excinuclease ABC, subunit A
TM0505	-2	11.8	groES protein
TM0506	-1	8.2	groEL protein
TM0562	-1	10.1	hypothetical protein
TM0570	-1.1	9.6	cell division protein FtsY
TM0571	-1.7	9.7	heat shock serine protease, periplasmic
TM0603	-1.7	5.3	ribosomal protein S6
TM0604	-2	14.1	single stranded DNA-binding protein, putative
TM0605	-1.9	15.6	ribosomal protein S18

TM0606	-1.4	14.6	hypothetical protein
TM0607	-1.2	11.5	hypothetical protein
TM0637	-1	12.8	hypothetical protein
TM0654	-2.6	12.9	spermidine synthase
TM0655	-3	32.6	conserved hypothetical protein
TM0656	-2.3	15.6	conserved hypothetical protein
TM0673	-1	15.4	basal-body rod modification protein FlgD
TM0676	-1.1	12.6	motility protein A
TM0677	-1.1	10.4	motility protein B
TM0758	-2.9	37.1	flagellin
TM0762	-1.3	6.2	ribosomal protein S2
TM0764	-1.1	14.6	hypothetical protein
TM0784	-1.4	14.8	hypothetical protein
TM0787	-1	3.1	thiamine biosynthetic enzyme
TM0814	-1	5.5	N-acetylglucosamine-6-phosphate deacetylase
TM0816	-1.2	10.5	transcriptional regulator, putative, Mar family
TM0818	-1.2	10.6	lipopolysaccharide biosynthesis protein, putative
TM0823	-1.6	13	transcriptional regulator, TetR family
TM0824	-1.1	6.9	astB/chuR-related protein
TM0927	-1.6	16.1	ferredoxin
TM0979	-1.1	11.9	conserved hypothetical protein
TM0980	-1.6	5.2	hypothetical protein
TM0981	-1.4	6.6	conserved hypothetical protein
TM0982	-1.5	9.9	conserved hypothetical protein
TM0983	-1.3	10	conserved hypothetical protein
TM0985	-1.1	9.1	hypothetical protein
TM1016	-1.2	4.4	hypothetical protein
TM1059	-1.2	11.9	spoVS-related protein
TM1064	-1	2.9	oligopeptide ABC transporter, ATP-binding protein
TM1068	-1.1	14	alpha-glucosidase, putative
TM1112	-1.8	11.5	hypothetical protein
TM1127	-1.6	6.9	hypothetical protein
TM1128	-1	2.3	ferritin
TM1135	-1.8	20.8	branched chain amino acid ABC transporter, periplasmic amino acid-binding protein
TM1143	-1.4	10	methyl-accepting chemotaxis protein
TM1182	-1	9.5	chromosome segregation SMC protein, putative
TM1195	-1	3.6	beta-galactosidase
TM1198	-1.1	6.6	oligopeptide ABC transporter, permease protein
TM1203	-1.1	8.2	maltose ABC transporter, permease protein
TM1204	-1.1	13	maltose ABC transporter, periplasmic maltose-binding protein
TM1215	-1.2	8	NADH dehydrogenase, 30 kDa subunit, putative
TM1227	-1.1	10.4	endo-1,4-beta-mannosidase
TM1271	-1.1	6.3	type IV pilin-related protein
TM1281	-1	3.3	6-phospho-beta-glucosidase
TM1344	-2.2	13.9	ribosomal protein S15
TM1374	-1	3.5	phosphoglycerate mutase
TM1411	-1.3	7.6	helicase-related protein

TM1451	-2.4	14.4	RNA polymerase sigma-A factor
TM1453	-2	11.6	ribosomal protein S9
TM1454	-1.4	28.2	ribosomal protein L13
TM1455	-1.3	4.1	conserved hypothetical protein
TM1456	-1.1	3.5	ribosomal protein L27
TM1457	-1.9	4.4	hypothetical protein
TM1458	-2.3	17.1	ribosomal protein L21
TM1484	-1	4.4	ribosomal protein L18
TM1485	-1.7	9.4	ribosomal protein L6
TM1488	-1	1.9	ribosomal protein L5
TM1489	-1.2	2.2	ribosomal protein L24
TM1491	-1.7	9.7	ribosomal protein S17
TM1492	-2.1	15.3	ribosomal protein L29
TM1493	-1.7	8.6	ribosomal protein L16
TM1494	-2.2	8.4	ribosomal protein S3
TM1495	-1.6	2.9	ribosomal protein L22
TM1497	-1.1	3.3	ribosomal protein L2
TM1498	-1.6	8.1	ribosomal protein L23
TM1499	-1.6	23.8	ribosomal protein L4
TM1504	-1.2	15	ribosomal protein S7
TM1565	-1	10.1	signal recognition particle protein
TM1568	-1.3	5.6	16S rRNA processing protein, putative
TM1569	-1	1.6	tRNA guanine-N1 methyltransferase
TM1570	-1.1	9.6	conserved hypothetical protein
TM1576	-1.2	12.3	hemolysin
TM1590	-1.5	12.6	translation initiation factor IF-3
TM1591	-1.7	10.1	ribosomal protein L35
TM1592	-1	2.1	ribosomal protein L20
TM1593	-1.2	3.4	conserved hypothetical protein
TM1598	-1.3	9.9	RNA polymerase sigma-E factor
TM1599	-1	5	hypothetical protein
TM1627	-1.2	6.9	general stress protein Ctc
TM1683	-2.3	17.1	cold shock protein
TM1706	-1.5	3.2	transcription elongation factor, greA/greB family
TM1707	-1	3.6	conserved hypothetical protein
TM1755	-1.3	9.5	phosphate butyryltransferase
TM1756	-1.5	7.8	branched-chain-fatty-acid kinase, putative
TM1760	-1.2	6.7	
TM1776	-1	7.8	ferric uptake regulation protein
TM1777	-2.2	12	N utilization substance protein A
TM1778	-2	9.3	conserved hypothetical protein
TM1835	-1	1.2	cyclomaltodextrinase, putative
TM1839	-1.3	17.7	maltose ABC transporter, periplasmic maltose-binding protein
TM1840	-1.5	9.8	alpha-amylase
TM1845	-1.1	11.1	pullulanase
TM1874	-1.8	12.5	cold shock protein

TABLE 2.8. Differentially expressed ORFs for *T. maritima* batch challenge WT05 vs. WT30			
7 ORFs up-regulated in WT at 5 + 29 ORFs up-regulated in WT at 30 min = 36 Total ORFs			
Gene	**Log(2) fold change WT05 vs. WT30**	**-log_{10}pval**	**Annotation**
TM0031	1.2	2.7	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0044	1.9	7.5	hypothetical protein
TM0050	1.4	3.1	iron(II) transport protein A
TM0266	1.6	5.1	DNA-binding protein, HU
TM0295	1.7	7.2	transaldolase-related protein
TM0338	1.3	7.3	hypothetical protein
TM0369	1	7.1	conserved hypothetical protein
TM0473	1.1	3.2	conserved hypothetical protein
TM0505	1.7	9.2	groES protein
TM0593	1	6.3	amino acid ABC transporter, periplasmic amino acid-binding protein
TM0688	1.9	12.9	glyceraldehyde-3-phosphate dehydrogenase
TM0689	2	9.1	phosphoglycerate kinase/triose-phosphate isomerase
TM0786	1.2	8.3	hypothetical protein
TM0894	1	10	conserved hypothetical protein
TM1112	1.2	6.7	hypothetical protein
TM1128	1.3	3.2	ferritin
TM1135	1.3	15.3	branched chain amino acid ABC transporter, periplasmic amino acid-binding protein
TM1223	1.7	12.8	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM1375	1	7.4	spermidine/putrescine ABC transporter, periplasmic spermidine/putrescine-binding protein
TM1400	1.6	18.8	aspartate aminotransferase, putative
TM1401	1.7	13.2	D-3-phosphoglycerate dehydrogenase
TM1456	1	2.8	ribosomal protein L27
TM1457	1.5	2.9	hypothetical protein
TM1458	1.2	7.1	ribosomal protein L21
TM1475	1.1	2.1	ribosomal protein S13
TM1505	1	1.5	ribosomal protein S12
TM1507	1	8.5	phoH-related protein
TM1571	1.1	1.6	ribosomal protein L19
TM1763	1	2.7	translation elongation factor P
TM_tRNA-Gln-2	-1.2	6	
TM0179	-1	8.9	hypothetical protein
TM0211	-1.2	14.3	aminomethyltransferase
TM0504	-2	21.4	hypothetical protein
TM0580	-1.1	6	cell division protein FtsH
TM0654	-1.3	4.7	spermidine synthase
TM0943	-1.3	12.2	glutamine synthetase

TABLE 2.9. Differentially expressed ORFs for *T. maritima* CC CAM challenge RM00 vs. WT00
7 ORFs up-regulated in RM + 12 ORFs up-regulated in WT = 19 Total ORFs

Gene	Log(2) fold change RM00 vs. WT00	$-\log_{10}$pval	Annotation
TM0185	1.0	3	hypothetical protein
TM0979	1.7	18.4	conserved hypothetical protein
TM0980	2.0	7.8	hypothetical protein
TM0981	1.7	8.9	conserved hypothetical protein
TM0982	1.4	8.6	conserved hypothetical protein
TM0983	1.1	19.6	conserved hypothetical protein
TM1849	1.5	16.4	hypothetical protein
TM0024	-1.9	31.9	laminarinase
TM0025	-1.3	8.6	beta-glucosidase
TM0028	-1.7	21.3	oligopeptide ABC transporter, ATP-binding protein
TM0029	-1.2	16.9	oligopeptide ABC transporter, permease protein
TM0030	-1.6	8.6	oligopeptide ABC transporter, permease protein
TM0031	-3.2	22.6	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0032	-1.7	25.6	transcriptional regulator, XylR-related
TM0402	-1.5	24	ammonium transporter
TM0403	-2.1	13.8	nitrogen regulatory protein P-II
TM0784	-1.0	5.8	hypothetical protein
TM0943	-1.8	18.1	glutamine synthetase
TM1301	-1.1	13.8	astB/chuR-related protein

TABLE 2.10. Differentially expressed ORFs for *T. maritima* CC CAM challenge RM05 vs. WT05

136 ORFs up-regulated in RM + 135 ORFs up-regulated in WT = 271 Total ORFs

ORF #	Log (2) fold change RM05 vs. WT05	$-\log_{10}$pval	Annotation
TM0009	1	1.7	hypothetical protein
TM0035	1.2	6.8	hypothetical protein
TM0040	1	11.4	dihydropteroate synthase
TM0074	1.2	4.7	oligopeptide ABC transporter, ATP-binding protein
TM0076	1.9	26.6	xylosidase
TM0077	1.5	3.8	acetyl xylan esterase
TM0088	1.2	10.7	comE protein, putative
TM0089	1.2	9.3	hypothetical protein
TM0090	1.8	14.3	hypothetical protein
TM0092	1.8	11.8	hypothetical protein
TM0096	1	7.3	conserved hypothetical protein
TM0103	1.4	10.2	sugar ABC transporter, ATP-binding protein
TM0104	1.1	7.4	sugar ABC transporter, permease protein
TM0110	1.4	16.6	transcriptional regulator, XylR-related
TM0113	1.2	9.1	xylU-related protein
TM0137	1.6	7.8	tryptophan synthase, alpha subunit
TM0138	1	5.4	tryptophan synthase, beta subunit
TM0139	1.3	12.2	phosphoribosylanthranilate isomerase
TM0140	1.3	3.1	indole-3-glycerol phosphate synthase
TM0171	1.1	8.4	conserved hypothetical protein
TM0172	1	5.1	adenosylhomocysteinase
TM0215	1.5	9	protein synthesis inhibitor, putative
TM0216	1.5	6.1	glycyl-tRNA synthetase, alpha subunit
TM0218	1.3	13.6	flagellum-specific ATP synthase
TM0219	1	7.2	flagellar export/assembly protein
TM0220	1	13.1	flagellar motor switch protein FliG
TM0261	1	2.8	phosphate permease, putative
TM0273	1.1	7.5	fructose-bisphosphate aldolase
TM0274	2.1	15.4	acetate kinase
TM0275	1.4	7.4	transcriptional regulator, GntR family
TM0313	1.4	15.9	K+ channel, beta subunit
TM0393	1.2	3.9	transcriptional regulator, XylR-related
TM0395	2.1	7	NADH oxidase, putative
TM0396	1.6	10.7	iron-sulfur cluster-binding protein
TM0405	1.1	9.3	keto/oxoacid ferredoxin oxidoreductase, beta subunit, putative
TM0410	1.2	7.2	conserved hypothetical protein
TM0427	1.6	21.7	oxidoreductase, putative
TM0446	1.3	13.3	phosphoribosylaminoimidazole carboxylase, catalytic subunit
TM0459	1.2	8.3	DNA-directed RNA polymerase, beta' subunit

TM0463	1.4	3.1	lipoprotein signal peptidase
TM0465	1.1	4.1	hypothetical protein
TM0501	1	5.6	oligopeptide ABC transporter, ATP-binding protein
TM0508	1.1	3.1	conserved hypothetical protein
TM0533	1.1	7	oligopeptide ABC transporter, permease protein
TM0549	1.5	8.9	acetolactate synthase, small subunit
TM0550	1.3	14.7	ketol-acid reductoisomerase
TM0555	1	4.2	3-isopropylmalate dehydratase, small subunit
TM0561	1.4	6.3	divalent cation transport-related protein
TM0624	1.1	7.5	N-acetylglucosaminyl-phosphatidylinositol biosynthesis-related protein
TM0627	1.7	4.6	lipopolysaccharide biosynthesis protein
TM0628	1	7.4	hypothetical protein
TM0630	1.8	22.2	nucleotide sugar epimerase, putative
TM0631	1.4	5.5	lipopolysaccharide biosynthesis protein
TM0640	1.1	7.6	conserved hypothetical protein
TM0641	1.1	13	hypothetical protein
TM0642	1.1	6.5	hypothetical protein
TM0687	2.2	10.9	conserved hypothetical protein
TM0691	1	3.5	conserved hypothetical protein
TM0697	1	9.5	flagellar biosynthesis protein FliQ
TM0698	1	1.7	flagellar biosynthesis protein FliP
TM0702	1.4	8	chemotaxis sensor histidine kinase CheA
TM0712	1	10.2	conserved hypothetical protein
TM0714	1.6	7.3	hypothetical protein
TM0715	1.2	5.5	tRNA nucleotidyl transferase-related protein
TM0717	1.4	9.7	propionyl-CoA carboxylase, gamma subunit
TM0720	1.4	4.4	serine hydroxymethyltransferase
TM0721	1	10.2	uracil phosphoribosyltransferase
TM0733	1.1	2.4	sigma-B regulator, putative
TM0742	1.3	12.9	serine/threonine protein phosphatase
TM0744	1.8	7.8	conserved hypothetical protein
TM0864	1.2	12.5	conserved hypothetical protein
TM0896	1.3	5.7	galactose-1-phosphate uridylyltransferase, putative
TM0921	1.1	12.1	hypothetical protein
TM0974	1.2	5.4	hypothetical protein
TM0979	1	6.9	conserved hypothetical protein
TM0980	1.2	2.7	hypothetical protein
TM0981	1.2	3.5	conserved hypothetical protein
TM0991	1	5.6	hypothetical protein
TM1078	1.3	5.4	conserved hypothetical protein
TM1081	1.5	17.8	anti-sigma factor antagonist, putative
TM1082	1.8	12.5	lexA repressor
TM1083	1.5	7.8	conserved hypothetical protein
TM1098	1.3	12.4	hypothetical protein
TM1168	1.9	11.8	
TM1193	1.3	3	beta-galactosidase
TM1200	1.3	3.4	transcriptional regulator, LacI family
TM1218	3	32	transcriptional regulator, LacI family

TM1219	2	6.3	oligopeptide ABC transporter, ATP-binding protein
TM1220	3.4	28.6	oligopeptide ABC transporter, ATP-binding protein
TM1221	2.6	9.4	oligopeptide ABC transporter, permease protein
TM1222	1.8	15.7	oligopeptide ABC transporter, permease protein
TM1245	2	18	phosphoribosylformylglycinamidine synthase I
TM1256	1	9.2	ABC transporter, ATP-binding protein
TM1259	1	3.9	phosphate regulon transcriptional regulatory protein PhoB
TM1260	1.3	5.3	phosphate transport system regulator PhoU
TM1273	1	6.6	glutamyl tRNA-Gln amidotransferase, subunit B
TM1274	1.8	20.8	hypothetical protein
TM1276	1.4	19.1	sugar ABC transporter, ATP-binding protein
TM1307	1	1.7	hypothetical protein
TM1319	1.2	16.6	ABC transporter, ATP-binding protein
TM1334	1.8	18.2	conserved hypothetical protein
TM1335	1.7	19.1	hypothetical protein
TM1345	1.6	4.2	polynucleotide phosphorylase
TM1347	1.2	6.2	inosine-5'-monophosphate dehydrogenase
TM1362	1.1	10.8	motility protein PilT
TM1363	1.5	3	peptide chain release factor RF-1
TM1380	1.3	2.9	conserved hypothetical protein
TM1381	1.9	14.6	hypothetical protein
TM1382	1.3	7.3	conserved hypothetical protein
TM1447	1	10.4	conserved hypothetical protein
TM1460	1.4	9	jag protein, putative
TM1461	1	2.1	inner membrane protein, putative
TM1468	1.9	14.3	conserved hypothetical protein
TM1469	1.4	15.3	glucokinase
TM1470	1.6	10.4	transcription termination factor Rho
TM1478	1.2	17.9	methionine aminopeptidase
TM1479	1.9	12.5	adenylate kinase
TM1480	1.3	8.6	preprotein translocase SecY subunit
TM1601	1.2	16.6	conserved hypothetical protein
TM1610	1.7	17.2	ATP synthase F1, subunit beta
TM1611	2	8.6	ATP synthase F1, subunit gamma
TM1612	1.3	12.5	ATP synthase F1, subunit alpha
TM1613	1.4	8.4	ATP synthase F1, subunit delta
TM1697	1	7.5	conserved hypothetical protein
TM1699	1.1	7	conserved hypothetical protein
TM1704	1.7	8	hypothetical protein
TM1716	1	3	hypothetical protein
TM1718	1	13	ribulose-phosphate 3-epimerase
TM1734	1.2	6.6	phosphate transport system regulator PhoU, putative
TM1767	1.4	7.9	methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase
TM1768	1.2	5.1	exodeoxyribonuclease VII, large subunit
TM1808	1.3	6.7	conserved hypothetical protein
TM1835	2.3	18.4	cyclomaltodextrinase, putative
TM1836	2.4	28.4	maltose ABC transporter, permease protein
TM1840	1	12.7	alpha-amylase

TM1847	1.4	6.2	ROK family protein
TM0015	-1.3	12.1	pyruvate ferredoxin oxidoreductase, gamma subunit
TM0016	-1.2	7.4	pyruvate ferredoxin oxidoreductase, delta subunit
TM0017	-1.2	6.3	pyruvate ferredoxin oxidoreductase, alpha subunit
TM0031	-3.2	19.3	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0044	-1.3	5.4	hypothetical protein
TM0048	-1.2	15	transposase
TM0080	-1.2	5.9	iron(III) ABC transporter, periplasmic-binding protein, putative
TM0107	-1	7	conserved hypothetical protein
TM0121	-1	7.3	conserved hypothetical protein
TM0144	-2	15.4	conserved hypothetical protein
TM0150	-1.1	5.2	ribosomal protein L32
TM0151	-1.3	11.3	conserved hypothetical protein
TM0152	-1	3.2	hypothetical protein
TM0160	-1.2	9.7	conserved hypothetical protein
TM0179	-2.1	21.4	hypothetical protein
TM0180	-1.5	15.1	hypothetical protein
TM0192	-1.4	8.6	spoVS-related protein
TM0195	-1	7.7	guanosine pentaphosphate phosphohydrolase, putative
TM0198	-2	9.8	ATP-dependent Clp protease, ATPase subunit
TM0225	-1.4	6.3	1-aminocyclopropane-1-carboxylate deaminase, putative
TM0239	-1.1	2	glucose-1-phosphate adenylyltransferase
TM0255	-1.1	6.1	ribosomal protein L28
TM0266	-1.6	7.5	DNA-binding protein, HU
TM0295	-2.2	6.6	transaldolase-related protein
TM0308	-1.8	17.6	alpha-xylosidase
TM0321	-1.2	6.2	hypothetical protein
TM0359	-1.1	7.5	conserved hypothetical protein
TM0369	-1.6	16.7	conserved hypothetical protein
TM0374	-1.1	10.2	heat shock protein, class I
TM0375	-1.8	10.1	hypothetical protein
TM0379	-1	3.4	NADH oxidase
TM0439	-1	1.8	transcriptional regulator, GntR family
TM0445	-1	3.3	conserved hypothetical protein
TM0451	-1.1	2.5	ribosomal protein L33
TM0472	-1.4	13.7	amidotransferase, putative
TM0473	-1.9	17.2	conserved hypothetical protein
TM0475	-1	8.5	pyrazinamidase/nicotinamidase-related protein
TM0505	-1.4	11.9	groES protein
TM0506	-1.7	15	groEL protein
TM0521	-1.5	15.6	heat shock protein HslV
TM0522	-1.1	12.3	heat shock protein HslU
TM0547	-1.8	18.5	aspartokinase II
TM0560	-1.1	7.8	conserved hypothetical protein
TM0603	-2.2	10.3	ribosomal protein S6
TM0604	-2.8	26	single stranded DNA-binding protein, putative
TM0605	-1.8	18.9	ribosomal protein S18

TM0606	-1.8	17.6	hypothetical protein
TM0654	-2.4	10.1	spermidine synthase
TM0655	-2.3	26.5	conserved hypothetical protein
TM0657	-1.5	5.3	rubrerythrin
TM0695	-1.6	23.1	ATP-dependent Clp protease, proteolytic subunit
TM0711	-1	5	hypothetical protein
TM0726	-1.9	16.6	tldD protein
TM0758	-2.3	15.3	flagellin
TM0762	-1.3	3.1	ribosomal protein S2
TM0775	-1	5.4	translation initiation factor IF-2
TM0784	-1.3	5.8	hypothetical protein
TM0785	-1	10.8	bacteriocin
TM0786	-1.3	14.9	hypothetical protein
TM0787	-1	3.2	thiamine biosynthetic enzyme
TM0877	-1	9.5	enolase
TM0882	-1.9	11.4	O-acetylhomoserine sulfhydrylase
TM0894	-1	4.9	conserved hypothetical protein
TM0943	-1.3	8.6	glutamine synthetase
TM0958	-1	6.3	ribose ABC transporter, periplasmic ribose-binding protein
TM1045	-1.3	11.4	conserved hypothetical protein
TM1047	-1	7.6	septum site-determining protein MinC, putative
TM1059	-1.7	22	spoVS-related protein
TM1064	-1	5.6	oligopeptide ABC transporter, ATP-binding protein
TM1069	-1.1	10	transcriptional regulator, DeoR family
TM1112	-1.8	21.3	hypothetical protein
TM1128	-1.4	3.9	ferritin
TM1135	-2	17.5	branched chain amino acid ABC transporter, periplasmic amino acid-binding protein
TM1143	-1.4	14.5	methyl-accepting chemotaxis protein
TM1145	-1	7.7	conserved hypothetical protein
TM1146	-1.5	15	methyl-accepting chemotaxis protein
TM1161	-1.6	15.4	Mg2+ transporter MgtE, putative
TM1162	-1.2	15.7	conserved hypothetical protein
TM1183	-1.5	10.6	oxidoreductase, aldo/keto reductase family
TM1241	-1.1	9.2	hypothetical protein
TM1266	-1.7	14.7	hypothetical protein
TM1271	-1.5	13.1	type IV pilin-related protein
TM1316	-1.6	5.4	hypothetical protein
TM1344	-1.4	10.8	ribosomal protein S15
TM1360	-1.2	14.6	response regulator
TM1366	-1	3.8	flagellar hook-basal body complex protein FliE
TM1368	-1	13.8	ABC transporter, ATP-binding protein
TM1374	-1.4	9	phosphoglycerate mutase
TM1391	-1	8.5	ATP-dependent Clp protease, ATPase subunit
TM1400	-1	9.8	aspartate aminotransferase, putative
TM1451	-1.5	7.2	RNA polymerase sigma-A factor
TM1453	-1.6	6.3	ribosomal protein S9
TM1454	-1.7	18.9	ribosomal protein L13
TM1455	-1.6	4.8	conserved hypothetical protein

TM1456	-1.4	16	ribosomal protein L27
TM1457	-2.1	9.5	hypothetical protein
TM1458	-1.9	7.1	ribosomal protein L21
TM1471	-1.6	19.7	ribosomal protein L17
TM1474	-1.1	10.3	ribosomal protein S11
TM1475	-1.1	3.4	ribosomal protein S13
TM1504	-1	5.4	ribosomal protein S7
TM1505	-1.7	21.1	ribosomal protein S12
TM1507	-1	7.8	phoH-related protein
TM1509	-1.7	21.4	conserved hypothetical protein
TM1524	-1.1	9.8	endoglucanase
TM1546	-1	2.4	single stranded DNA-specific exonuclease, putative
TM1551	-1.2	4.2	conserved hypothetical protein
TM1565	-1.8	18.9	signal recognition particle protein
TM1566	-1.2	4.4	ribosomal protein S16
TM1567	-1	7.5	conserved hypothetical protein
TM1568	-1.6	8.5	16S rRNA processing protein, putative
TM1570	-1.1	4.4	conserved hypothetical protein
TM1571	-1.1	1.7	ribosomal protein L19
TM1572	-1.3	5.3	signal peptidase I, putative
TM1590	-1.5	15.8	translation initiation factor IF-3
TM1591	-1.9	21.2	ribosomal protein L35
TM1592	-1.5	10.2	ribosomal protein L20
TM1605	-1.1	10.6	translation elongation factor Ts
TM1627	-1.6	6.6	general stress protein Ctc
TM1683	-1.6	24.3	cold shock protein
TM1706	-1	2.3	transcription elongation factor, greA/greB family
TM1707	-1.4	13.2	conserved hypothetical protein
TM1720	-1.1	6.2	conserved hypothetical protein
TM1763	-1.1	2.4	translation elongation factor P
TM1765	-1.1	4.5	N utilization substance protein B
TM1776	-1.3	15.8	ferric uptake regulation protein
TM1777	-2.4	6.8	N utilization substance protein A
TM1778	-1.6	6.6	conserved hypothetical protein
TM1786	-1.5	5.6	hypothetical protein
TM1803	-1	11.2	dnaJ-related protein
TM1820	-1.2	2.6	GMP synthase
TM1825	-1.3	13.8	6,7-dimethyl-8-ribityllumazine synthase
TM1852	-1	11	conserved hypothetical protein
TM1874	-1.4	20.8	cold shock protein
TM1878	-1.7	12.5	UDP-sugar hydrolase

TABLE 2.11. Differentially expressed ORFs for *T. maritima* CC challenge RM30 vs. WT30

74 ORFs up-regulated in RM + 80 ORFs up-regulated in WT = 154 Total ORFs

ORF #	Log(2) fold change RM30 vs. WT30	$-\log_{10}$pval	Annotation
TM0076	1.6	28.9	xylosidase
TM0077	1.2	4.2	acetyl xylan esterase
TM0088	1	12.5	comE protein, putative
TM0089	1.1	12.5	hypothetical protein
TM0090	1.4	14.1	hypothetical protein
TM0092	1.4	11.8	hypothetical protein
TM0103	1.1	10.9	sugar ABC transporter, ATP-binding protein
TM0104	1	9.4	sugar ABC transporter, permease protein
TM0110	1.3	19.8	transcriptional regulator, XylR-related
TM0111	1.1	11.9	alcohol dehydrogenase, iron-containing
TM0112	1	6.7	sugar ABC transporter, permease protein
TM0113	1.2	12.2	xylU-related protein
TM0114	1	5.3	sugar ABC transporter, periplasmic sugar-binding protein
TM0215	1.3	10.5	protein synthesis inhibitor, putative
TM0216	1.2	6.8	glycyl-tRNA synthetase, alpha subunit
TM0273	1.1	10.2	fructose-bisphosphate aldolase
TM0274	1.8	17.6	acetate kinase
TM0275	1.7	12.6	transcriptional regulator, GntR family
TM0313	1.4	20.6	K+ channel, beta subunit
TM0332	1.1	15.9	orotidine 5'-phosphate decarboxylase, putative
TM0381	1	10.2	dihydrolipoamide dehydrogenase
TM0446	1	13.6	phosphoribosylaminoimidazole carboxylase, catalytic subunit
TM0476	1.1	17.4	hypothetical protein
TM0505	1.8	21.3	groES protein
TM0506	1.6	20	groEL protein
TM0583	1	14.6	lipopolysaccharide biosynthesis protein
TM0627	1	3	lipopolysaccharide biosynthesis protein
TM0630	1.5	24	nucleotide sugar epimerase, putative
TM0631	1	4.2	lipopolysaccharide biosynthesis protein
TM0689	1.4	13.8	phosphoglycerate kinase/triose-phosphate isomerase
TM0697	1	14.5	flagellar biosynthesis protein FliQ
TM0767	1	11.8	maltodextrin glycosyltransferase
TM0980	1.2	3.4	hypothetical protein
TM0981	1.4	6.6	conserved hypothetical protein
TM0982	1	5.5	conserved hypothetical protein
TM0983	1.1	20.3	conserved hypothetical protein
TM0989	1.3	15.8	conserved hypothetical protein
TM1098	1.1	9.6	hypothetical protein
TM1168	2.8	23.8	
TM1201	1	11.2	arabinogalactan endo-1,4-beta-galactosidase, putative

TM1204	1	10	maltose ABC transporter, periplasmic maltose-binding protein
TM1218	3	38.3	transcriptional regulator, LacI family
TM1219	1.8	8.1	oligopeptide ABC transporter, ATP-binding protein
TM1220	2.7	30.2	oligopeptide ABC transporter, ATP-binding protein
TM1221	2	8.1	oligopeptide ABC transporter, permease protein
TM1222	1.8	22	oligopeptide ABC transporter, permease protein
TM1223	2.3	29.8	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM1245	1.7	15.3	phosphoribosylformylglycinamidine synthase I
TM1259	1	5.3	phosphate regulon transcriptional regulatory protein PhoB
TM1260	1	5.3	phosphate transport system regulator PhoU
TM1274	1.9	28.1	hypothetical protein
TM1276	2.1	34.7	sugar ABC transporter, ATP-binding protein
TM1328	1	14	ABC transporter, ATP-binding protein
TM1334	1.1	12.4	conserved hypothetical protein
TM1335	1	14.1	hypothetical protein
TM1363	1	2.3	peptide chain release factor RF-1
TM1381	1.5	15.4	hypothetical protein
TM1382	1	7.3	conserved hypothetical protein
TM1468	1.7	16.8	conserved hypothetical protein
TM1469	1.3	19.1	glucokinase
TM1470	1.5	13.3	transcription termination factor Rho
TM1478	1.1	20.9	methionine aminopeptidase
TM1479	1.4	10.4	adenylate kinase
TM1767	1.3	10.5	methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase
TM1768	1.1	4.7	exodeoxyribonuclease VII, large subunit
TM1808	1.6	12.5	conserved hypothetical protein
TM1809	1.3	4	conserved hypothetical protein
TM1810	1.4	18.1	hypothetical protein
TM1835	2.3	23.2	cyclomaltodextrinase, putative
TM1836	2.4	34.5	maltose ABC transporter, permease protein
TM1837	1.1	14.6	
TM1839	3.5	42.8	maltose ABC transporter, periplasmic maltose-binding protein
TM1840	1.8	29.3	alpha-amylase
TM1847	1.5	7.4	ROK family protein
TM0015	-1.3	14.3	pyruvate ferredoxin oxidoreductase, gamma subunit
TM0016	-1.3	9.2	pyruvate ferredoxin oxidoreductase, delta subunit
TM0017	-1.3	10.7	pyruvate ferredoxin oxidoreductase, alpha subunit
TM0048	-1	15.6	transposase
TM0144	-1.3	12.3	conserved hypothetical protein
TM0150	-1.2	8.5	ribosomal protein L32
TM0179	-1.5	19.7	hypothetical protein
TM0192	-1.2	10	spoVS-related protein
TM0198	-1.6	9.1	ATP-dependent Clp protease, ATPase subunit
TM0199	-1	7.9	DNA repair protein
TM0209	-1.1	2.7	6-phosphofructokinase

TM0295	-1.9	6.9	transaldolase-related protein
TM0375	-2.2	17.2	hypothetical protein
TM0398	-1	7.4	conserved hypothetical protein
TM0445	-1	4.4	conserved hypothetical protein
TM0455	-1.2	10.3	ribosomal protein L1
TM0457	-1.2	11.4	ribosomal protein L7/L12
TM0472	-1.2	14.8	amidotransferase, putative
TM0538	-1	9.4	cation efflux system protein
TM0547	-1.3	17.9	aspartokinase II
TM0558	-1	4.2	carbamoyl-phosphate synthetase, small subunit
TM0575	-1.2	9.4	crossover junction endodeoxyribonuclease
TM0603	-1.2	6	ribosomal protein S6
TM0604	-1.8	22.4	single stranded DNA-binding protein, putative
TM0605	-1.2	14.6	ribosomal protein S18
TM0606	-1.6	21.1	hypothetical protein
TM0654	-2.8	15	spermidine synthase
TM0655	-2.2	30.5	conserved hypothetical protein
TM0711	-1	7	hypothetical protein
TM0755	-1.2	15.4	conserved hypothetical protein
TM0762	-1	2.6	ribosomal protein S2
TM0784	-1.3	8	hypothetical protein
TM0788	-1	10.5	thiamine biosynthesis protein, putative
TM0816	-1.1	10.9	transcriptional regulator, putative, Mar family
TM0823	-1.5	6.6	transcriptional regulator, TetR family
TM0824	-1.1	6.5	astB/chuR-related protein
TM0826	-1	3.1	hypothetical protein
TM0882	-1.1	8.1	O-acetylhomoserine sulfhydrylase
TM0943	-1.2	11.3	glutamine synthetase
TM1045	-1.1	9.4	conserved hypothetical protein
TM1064	-1.4	12.6	oligopeptide ABC transporter, ATP-binding protein
TM1112	-2.3	31.3	hypothetical protein
TM1143	-1.6	20.5	methyl-accepting chemotaxis protein
TM1144	-1.2	10.9	hypothetical protein
TM1145	-1.8	20.6	conserved hypothetical protein
TM1146	-1.3	17.6	methyl-accepting chemotaxis protein
TM1161	-1.5	18.4	Mg2+ transporter MgtE, putative
TM1241	-1.6	17.8	hypothetical protein
TM1266	-1.7	18.1	hypothetical protein
TM1267	-2.2	13.3	thiH protein, putative
TM1268	-1	10.3	hypothetical protein
TM1344	-1.6	17	ribosomal protein S15
TM1374	-1.8	17.2	phosphoglycerate mutase
TM1453	-1.4	7.9	ribosomal protein S9
TM1454	-1.2	17.4	ribosomal protein L13
TM1455	-1.1	3.8	conserved hypothetical protein
TM1457	-1.2	5.1	hypothetical protein
TM1462	-1.1	13.2	conserved hypothetical protein
TM1463	-1.5	24.9	ribonuclease P protein component
TM1471	-1	16.3	ribosomal protein L17

TM1505	-1.2	19.8	ribosomal protein S12
TM1509	-2	29.8	conserved hypothetical protein
TM1565	-1.6	21.8	signal recognition particle protein
TM1570	-1	5.9	conserved hypothetical protein
TM1571	-1.1	1.7	ribosomal protein L19
TM1572	-1.2	6	signal peptidase I, putative
TM1591	-1.2	17.8	ribosomal protein L35
TM1592	-1.2	10.5	ribosomal protein L20
TM1593	-1.1	7.7	conserved hypothetical protein
TM1627	-1.2	6.2	general stress protein Ctc
TM1658	-1	9.9	S-adenosylmethionine synthetase
TM1706	-1.2	4.4	transcription elongation factor, greA/greB family
TM1707	-1.7	18.9	conserved hypothetical protein
TM1776	-1.8	28.4	ferric uptake regulation protein
TM1777	-1.5	4.8	N utilization substance protein A
TM1778	-1	4.3	conserved hypothetical protein
TM1782	-1	13.6	N-acetyl-gamma-glutamyl-phosphate reductase
TM1820	-1.1	3.3	GMP synthase
TM1852	-1	14.1	conserved hypothetical protein
TM1874	-1.6	29.9	cold shock protein

TABLE 2.12. Differentially expressed ORFs for *T. maritima* CC challenge RM00 vs. RM05			
1 ORFs up-regulated in RM at 0 + 7 ORFs up-regulated in RM at 5 min = 8 Total ORFs			
ORF #	**Log(2) fold change RM0 vs. RM05**	**$-\log_{10}$pval**	**Annotation**
TM1316	1.1	4.8	hypothetical protein
TM_tRNA-Gln-2	-1.2	9.8	
TM0211	-1.2	13.7	aminomethyltransferase
TM0269	-1.4	8.6	hypothetical protein
TM0319	-1.3	14.7	hypothetical protein
TM0506	-1.1	12.6	groEL protein
TM1266	-1.1	12	hypothetical protein
TM1267	-1.6	9	thiH protein, putative

TABLE 2.13. Differentially expressed ORFs for *T. maritima* CC challenge RM05 vs.RM30

ORF #	Log(2) fold change RM05 vs. RM30	$-\log_{10}$pval	Annotation
0 ORFs up-regulated in RM at 5 min + 5 ORFs down-regulated in RM at 5 min = 5 Total ORFs			
TM0050	-1	9.6	iron(II) transport protein A
TM0051	-1.7	19.4	iron(II) transport protein B
TM0403	-1.2	7.1	nitrogen regulatory protein P-II
TM0560	-1	10	conserved hypothetical protein
TM1267	-1.1	4.8	thiH protein, putative

TABLE 2.14. Differentially expressed ORFs for *T. maritima* CC CAM challenge WT00 vs. WT05			
125 ORFs up-regulated in WT at 0 + 131 ORFs down-regulated in WT at 5 min = 256 Total ORFs			
ORF #	**Log(2) fold change WT00 vs. WT05**	**$-\log_{10}$pval**	**Annotation**
TM0024	1.8	30.2	laminarinase
TM0025	1.9	13.5	beta-glucosidase
TM0028	1.7	21.5	oligopeptide ABC transporter, ATP-binding protein
TM0030	1.1	5	oligopeptide ABC transporter, permease protein
TM0032	1	16	transcriptional regulator, XylR-related
TM0035	1.7	16.4	hypothetical protein
TM0040	1	16.6	dihydropteroate synthase
TM0074	1.3	7.2	oligopeptide ABC transporter, ATP-binding protein
TM0076	1.1	22.5	xylosidase
TM0078	1.2	11.3	iron(III) ABC transporter, ATP-binding protein
TM0088	1.2	15.1	comE protein, putative
TM0090	1.8	19.5	hypothetical protein
TM0092	1.4	12	hypothetical protein
TM0094	1	9.6	general secretion pathway protein F, putative
TM0103	1.1	11.4	sugar ABC transporter, ATP-binding protein
TM0110	1.1	17.6	transcriptional regulator, XylR-related
TM0137	1.5	8.8	tryptophan synthase, alpha subunit
TM0138	1.3	11.9	tryptophan synthase, beta subunit
TM0139	1.6	20.6	phosphoribosylanthranilate isomerase
TM0140	1.7	6.2	indole-3-glycerol phosphate synthase
TM0147	1.1	16.7	conserved hypothetical protein
TM0169	1.3	9.4	conserved hypothetical protein
TM0171	1	7.6	conserved hypothetical protein
TM0215	1.2	9.4	protein synthesis inhibitor, putative
TM0216	1.2	6.7	glycyl-tRNA synthetase, alpha subunit
TM0218	1.4	18.3	flagellum-specific ATP synthase
TM0219	1.4	14.4	flagellar export/assembly protein
TM0220	1.2	20.9	flagellar motor switch protein FliG
TM0229	1	6	conserved hypothetical protein
TM0264	1	9.2	16S pseudouridylate synthase
TM0273	1.2	11.8	fructose-bisphosphate aldolase
TM0274	1.9	18.9	acetate kinase
TM0275	1.3	8.9	transcriptional regulator, GntR family
TM0313	1.6	22.7	K+ channel, beta subunit
TM0364	1	13	4-alpha-glucanotransferase
TM0365	1	2.2	aminopeptidase, putative
TM0393	1.2	5	transcriptional regulator, XylR-related
TM0395	1.6	6.3	NADH oxidase, putative
TM0396	1.3	10.1	iron-sulfur cluster-binding protein
TM0403	1.4	8.4	nitrogen regulatory protein P-II
TM0410	1.3	10.7	conserved hypothetical protein

TM0427	1.6	26	oxidoreductase, putative
TM0446	1.2	16.1	phosphoribosylaminoimidazole carboxylase, catalytic subunit
TM0459	1.1	11	DNA-directed RNA polymerase, beta' subunit
TM0463	1.5	4.4	lipoprotein signal peptidase
TM0495	1	11.2	phoH-related protein
TM0501	1	7.9	oligopeptide ABC transporter, ATP-binding protein
TM0513	1	8.8	comM protein
TM0532	1.3	7.7	oligopeptide ABC transporter, permease protein
TM0533	1.3	12.2	oligopeptide ABC transporter, permease protein
TM0549	2.2	19.1	acetolactate synthase, small subunit
TM0550	1.9	26.8	ketol-acid reductoisomerase
TM0553	1.4	6.2	2-isopropylmalate synthase
TM0554	1.2	10.6	3-isopropylmalate dehydratase, large subunit
TM0555	1.2	7.9	3-isopropylmalate dehydratase, small subunit
TM0624	1	10.2	N-acetylglucosaminyl-phosphatidylinositol biosynthesis-related protein
TM0630	1.8	28.1	nucleotide sugar epimerase, putative
TM0631	1.2	5.3	lipopolysaccharide biosynthesis protein
TM0640	1	9.8	conserved hypothetical protein
TM0641	1.1	18.6	hypothetical protein
TM0642	1	8.3	hypothetical protein
TM0687	2.5	17.2	conserved hypothetical protein
TM0702	2	16.2	chemotaxis sensor histidine kinase CheA
TM0712	1.4	18.9	conserved hypothetical protein
TM0714	1.8	11.8	hypothetical protein
TM0717	1.2	9.5	propionyl-CoA carboxylase, gamma subunit
TM0720	1.1	4.5	serine hydroxymethyltransferase
TM0733	1.1	3.8	sigma-B regulator, putative
TM0742	1.2	16.6	serine/threonine protein phosphatase
TM0744	1.5	8.3	conserved hypothetical protein
TM0753	1	11.1	ubiquinone/menaquinone biosynthesis methyltransferase, putative
TM0801	1.2	12.2	(3R)-hydroxymyristoyl-(acyl carrier protein) dehydratase
TM0804	1	8.7	conserved hypothetical protein
TM0864	1.5	22.2	conserved hypothetical protein
TM0880	1	8.2	oxaloacetate decarboxylase, beta subunit
TM0886	1	13.9	penicillin-binding protein, class 1A
TM0896	1.4	8.1	galactose-1-phosphate uridylyltransferase, putative
TM0902	1	2.9	RNA polymerase sigma-28 factor, putative
TM0904	1.1	12.9	chemotaxis protein CheC
TM0905	1.1	10.3	hypothetical protein
TM0921	1	16.1	hypothetical protein
TM0991	1.4	13.4	hypothetical protein
TM1081	1.2	18.4	anti-sigma factor antagonist, putative
TM1082	1.8	17.2	lexA repressor
TM1083	2	14.7	conserved hypothetical protein
TM1098	1.4	13.2	hypothetical protein
TM1218	2.4	33.2	transcriptional regulator, LacI family

TM1219	1.5	5.9	oligopeptide ABC transporter, ATP-binding protein
TM1220	3	32.6	oligopeptide ABC transporter, ATP-binding protein
TM1221	1.8	7.1	oligopeptide ABC transporter, permease protein
TM1222	1.2	14.3	oligopeptide ABC transporter, permease protein
TM1245	1.6	14.2	phosphoribosylformylglycinamidine synthase I
TM1256	1.2	15.8	ABC transporter, ATP-binding protein
TM1273	1.4	15.5	glutamyl tRNA-Gln amidotransferase, subunit B
TM1274	1.8	26.7	hypothetical protein
TM1301	1.3	16	astB/chuR-related protein
TM1319	1.5	25.9	ABC transporter, ATP-binding protein
TM1334	2	22.7	conserved hypothetical protein
TM1335	1.7	23.7	hypothetical protein
TM1345	1.8	6.2	polynucleotide phosphorylase
TM1346	1	10.8	processing protease, putative
TM1362	1.5	20.3	motility protein PilT
TM1363	1.3	3.7	peptide chain release factor RF-1
TM1380	1.3	4.8	conserved hypothetical protein
TM1381	1.7	17.8	hypothetical protein
TM1382	1.3	10.3	conserved hypothetical protein
TM1383	1	6.7	conserved hypothetical protein
TM1422	1.3	17.9	rnfB-related protein
TM1426	1.3	16.8	Fe-hydrogenase, subunit alpha
TM1427	1.1	13	conserved hypothetical protein
TM1442	1	13.1	anti-sigma factor antagonist, putative
TM1447	1	10.2	conserved hypothetical protein
TM1460	1.5	13.7	jag protein, putative
TM1468	1.7	17.2	conserved hypothetical protein
TM1469	1.3	20	glucokinase
TM1470	1.7	15.6	transcription termination factor Rho
TM1479	1.3	9.5	adenylate kinase
TM1545	1.2	10.2	conserved hypothetical protein
TM1548	1.2	18.3	lipopolysaccharide biosynthesis protein
TM1562	1	12.5	hypothetical protein
TM1610	1.7	22.7	ATP synthase F1, subunit beta
TM1611	2.2	13.7	ATP synthase F1, subunit gamma
TM1612	1.3	17	ATP synthase F1, subunit alpha
TM1699	1.3	12.3	conserved hypothetical protein
TM1704	1.4	8.5	hypothetical protein
TM1767	1.5	11.9	methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase
TM1808	1.3	9.7	conserved hypothetical protein
TM1809	1.2	3.4	conserved hypothetical protein
TM1810	1	12.4	hypothetical protein
TM1835	2.1	21.5	cyclomaltodextrinase, putative
TM1836	2.2	32.5	maltose ABC transporter, permease protein
TM_tRNA-Gln-2	-1.3	11	
TM0015	-1.2	12.7	pyruvate ferredoxin oxidoreductase, gamma subunit
TM0016	-1.1	7.2	pyruvate ferredoxin oxidoreductase, delta subunit
TM0044	-2.2	11.1	hypothetical protein

TM0071	-1	4.2	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0080	-1.4	10	iron(III) ABC transporter, periplasmic-binding protein, putative
TM0144	-1.7	16.4	conserved hypothetical protein
TM0152	-1	3.3	hypothetical protein
TM0160	-1.2	12.4	conserved hypothetical protein
TM0179	-2.1	26.9	hypothetical protein
TM0180	-1.6	21.3	hypothetical protein
TM0186	-1.2	14.3	response regulator
TM0192	-1.8	16.1	spoVS-related protein
TM0195	-1.1	11.8	guanosine pentaphosphate phosphohydrolase, putative
TM0198	-1.4	7	ATP-dependent Clp protease, ATPase subunit
TM0209	-1	2.4	6-phosphofructokinase
TM0211	-1.5	17.2	aminomethyltransferase
TM0266	-1.7	11.1	DNA-binding protein, HU
TM0269	-1.2	7.5	hypothetical protein
TM0295	-1.9	6.9	transaldolase-related protein
TM0308	-1.4	18.3	alpha-xylosidase
TM0319	-1.1	11.9	hypothetical protein
TM0369	-1.3	17.3	conserved hypothetical protein
TM0374	-1	13.6	heat shock protein, class I
TM0375	-1.6	12.4	hypothetical protein
TM0379	-1.2	6.4	NADH oxidase
TM0423	-1.3	5.5	glycerol dehydrogenase
TM0440	-1.1	5.7	hypothetical protein
TM0445	-1.3	6.8	conserved hypothetical protein
TM0472	-1.3	16.7	amidotransferase, putative
TM0473	-1.9	21.8	conserved hypothetical protein
TM0477	-1	3.9	outer membrane protein alpha
TM0505	-1.8	21.3	groES protein
TM0506	-2.2	25.4	groEL protein
TM0521	-1.1	15.8	heat shock protein HslV
TM0547	-1	13.1	aspartokinase II
TM0571	-1.5	7.1	heat shock serine protease, periplasmic
TM0603	-1.8	10.2	ribosomal protein S6
TM0604	-2.5	29.1	single stranded DNA-binding protein, putative
TM0605	-1.9	23.8	ribosomal protein S18
TM0606	-1.8	23.1	hypothetical protein
TM0607	-1	12.3	hypothetical protein
TM0654	-2.3	12.3	spermidine synthase
TM0655	-2.2	30.6	conserved hypothetical protein
TM0657	-1.6	7.9	rubrerythrin
TM0658	-1.2	11.8	neelaredoxin
TM0695	-1.7	29.6	ATP-dependent Clp protease, proteolytic subunit
TM0726	-1.1	11.7	tldD protein
TM0758	-2.7	22.9	flagellin
TM0762	-1.4	4.3	ribosomal protein S2
TM0785	-1.1	17.2	bacteriocin

TM0786	-1	14.9	hypothetical protein
TM0787	-1	4.8	thiamine biosynthetic enzyme
TM0824	-1.1	5.6	astB/chuR-related protein
TM0882	-1.5	11.8	O-acetylhomoserine sulfhydrylase
TM0979	-1.1	10.7	conserved hypothetical protein
TM0980	-1.6	5.5	hypothetical protein
TM0981	-1	3.9	conserved hypothetical protein
TM0982	-1.1	6.1	conserved hypothetical protein
TM0983	-1.4	23.9	conserved hypothetical protein
TM1045	-1	8.2	conserved hypothetical protein
TM1059	-1.5	25.5	spoVS-related protein
TM1069	-1.1	14.4	transcriptional regulator, DeoR family
TM1112	-1.5	23.2	hypothetical protein
TM1128	-1.1	3.7	ferritin
TM1135	-2	22.9	branched chain amino acid ABC transporter, periplasmic amino acid-binding protein
TM1143	-1.5	18.9	methyl-accepting chemotaxis protein
TM1145	-1	10.3	conserved hypothetical protein
TM1146	-1.2	15.6	methyl-accepting chemotaxis protein
TM1161	-1.3	15.9	Mg2+ transporter MgtE, putative
TM1183	-1.5	13.8	oxidoreductase, aldo/keto reductase family
TM1241	-1	10.7	hypothetical protein
TM1255	-1	11.4	aspartate aminotransferase
TM1266	-2.8	28.4	hypothetical protein
TM1267	-1.6	8.4	thiH protein, putative
TM1271	-1.6	18.3	type IV pilin-related protein
TM1344	-1.1	10.5	ribosomal protein S15
TM1366	-1	5	flagellar hook-basal body complex protein FliE
TM1368	-1.2	22.2	ABC transporter, ATP-binding protein
TM1451	-1.9	14	RNA polymerase sigma-A factor
TM1453	-1.8	10.6	ribosomal protein S9
TM1454	-2.2	29.2	ribosomal protein L13
TM1455	-1.9	8.6	conserved hypothetical protein
TM1456	-1.5	19.5	ribosomal protein L27
TM1457	-2.2	12.6	hypothetical protein
TM1458	-2.1	12	ribosomal protein L21
TM1471	-1.7	25.4	ribosomal protein L17
TM1474	-1	12.1	ribosomal protein S11
TM1475	-1.1	4.2	ribosomal protein S13
TM1485	-1.4	4.7	ribosomal protein L6
TM1488	-1.1	6.3	ribosomal protein L5
TM1492	-1.1	14.4	ribosomal protein L29
TM1493	-1.2	11.7	ribosomal protein L16
TM1496	-1.1	11.8	ribosomal protein S19
TM1504	-1.2	9.3	ribosomal protein S7
TM1505	-1.5	24.2	ribosomal protein S12
TM1509	-1.4	23.4	conserved hypothetical protein
TM1525	-1.1	12.6	endoglucanase
TM1551	-1.1	5.2	conserved hypothetical protein

TM1565	-2.1	26.9	signal recognition particle protein
TM1566	-1.1	5.1	ribosomal protein S16
TM1567	-1.3	13.7	conserved hypothetical protein
TM1568	-1.7	13	16S rRNA processing protein, putative
TM1569	-1.2	1.9	tRNA guanine-N1 methyltransferase
TM1571	-1.3	2.2	ribosomal protein L19
TM1572	-1.5	7.9	signal peptidase I, putative
TM1590	-2	26.6	translation initiation factor IF-3
TM1591	-2.4	31.3	ribosomal protein L35
TM1592	-1.8	17.4	ribosomal protein L20
TM1593	-1.3	9.9	conserved hypothetical protein
TM1605	-1.1	14	translation elongation factor Ts
TM1627	-1.8	10.9	general stress protein Ctc
TM1628	-1.1	13.3	phosphoribosyl pyrophosphate synthetase
TM1666	-1	5.3	succinyl-diaminopimelate desuccinylase, putative
TM1683	-1.4	27.3	cold shock protein
TM1706	-1.6	6.8	transcription elongation factor, greA/greB family
TM1707	-1.5	16.3	conserved hypothetical protein
TM1776	-1.6	26.3	ferric uptake regulation protein
TM1777	-2.5	9.5	N utilization substance protein A
TM1778	-1.8	9.6	conserved hypothetical protein
TM1825	-1.2	17.5	6,7-dimethyl-8-ribityllumazine synthase
TM1852	-1.4	20.3	conserved hypothetical protein
TM1857	-1	8.5	conserved hypothetical protein
TM1874	-1.7	30.7	cold shock protein
TM1878	-1.4	12.8	UDP-sugar hydrolase

TABLE 2.15. Differentially expressed ORFs for *T. maritima* CC challenge WT05 vs. WT30

32 ORFs up-regulated in WT at 5 min + 47 ORFs up-regulated in WT at 30 min = 79 Total ORFs

ORF #	Log (2) fold change WT05 vs. WT30	$-\log_{10}$pval	Annotation
TM0199	1.1	8.4	DNA repair protein
TM0385	1.1	5.4	conserved hypothetical protein
TM0395	1.7	6.9	NADH oxidase, putative
TM0396	1.4	10.8	iron-sulfur cluster-binding protein
TM0397	1.1	7.1	glutamate synthase, alpha subunit
TM0456	1.3	7.6	ribosomal protein L10
TM0457	1.8	17.9	ribosomal protein L7/L12
TM0548	1	9.5	acetolactate synthase, large subunit
TM0549	1.5	12.8	acetolactate synthase, small subunit
TM0550	1.6	23.7	ketol-acid reductoisomerase
TM0576	1.1	8.8	DNA polymerase III, alpha subunit
TM0687	1.3	7.7	conserved hypothetical protein
TM0712	1.2	16.1	conserved hypothetical protein
TM0713	1	3.2	hypothetical protein
TM0714	1.2	7.2	hypothetical protein
TM0823	1.5	6.6	transcriptional regulator, TetR family
TM1083	1	6.4	conserved hypothetical protein
TM1239	1.1	2.7	conserved hypothetical protein
TM1267	1	4.3	thiH protein, putative
TM1345	1.1	2.8	polynucleotide phosphorylase
TM1424	1	16.1	Fe-hydrogenase, subunit gamma
TM1462	1.2	15.1	conserved hypothetical protein
TM1495	1	2.3	ribosomal protein L22
TM1497	1.3	2.5	ribosomal protein L2
TM1498	1.3	6.5	ribosomal protein L23
TM1499	1.3	21.5	ribosomal protein L4
TM1500	1.1	4.2	ribosomal protein L3
TM1501	1.1	11	ribosomal protein S10
TM1611	1.4	6.8	ATP synthase F1, subunit gamma
TM1658	1.2	12.8	S-adenosylmethionine synthetase
TM1704	1.6	10.2	hypothetical protein
TM1705	1.5	19.1	lysyl-tRNA synthetase
TM0031	-2.5	18	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM0044	-2	9.9	hypothetical protein
TM0050	-1.4	13.8	iron(II) transport protein A
TM0051	-1.2	13.7	iron(II) transport protein B
TM0144	-1	8.3	conserved hypothetical protein
TM0160	-1.4	14.2	conserved hypothetical protein
TM0180	-1.5	19.6	hypothetical protein
TM0266	-1	5.3	DNA-binding protein, HU

TM0308	-1.6	21.1	alpha-xylosidase
TM0369	-2.3	29.2	conserved hypothetical protein
TM0374	-1.5	20.3	heat shock protein, class I
TM0402	-1.1	18.7	ammonium transporter
TM0403	-2.4	16.7	nitrogen regulatory protein P-II
TM0439	-1	2.8	transcriptional regulator, GntR family
TM0460	-1.6	23	oligopeptide ABC transporter, periplasmic oligo-peptide binding protein, putative
TM0476	-1.6	23.3	hypothetical protein
TM0477	-1	4.5	outer membrane protein alpha
TM0505	-2.5	28	groES protein
TM0506	-2.6	29.5	groEL protein
TM0521	-1.1	15.3	heat shock protein HslV
TM0560	-1.7	18.6	conserved hypothetical protein
TM0726	-1.2	14	tldD protein
TM0758	-1.8	15.5	flagellin
TM0785	-1.3	20.1	bacteriocin
TM0786	-1.7	25.9	hypothetical protein
TM0830	-1	13.4	conserved hypothetical protein
TM0894	-1	7.1	conserved hypothetical protein
TM0897	-1	10.6	spoVS-related protein
TM0989	-1.6	20.4	conserved hypothetical protein
TM1059	-2	31.8	spoVS-related protein
TM1127	-1	3.7	hypothetical protein
TM1135	-1.6	18.4	branched chain amino acid ABC transporter, periplasmic amino acid-binding protein
TM1162	-1.1	18.3	conserved hypothetical protein
TM1204	-1	9.7	maltose ABC transporter, periplasmic maltose-binding protein
TM1223	-2.6	32.5	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein
TM1271	-2	22.9	type IV pilin-related protein
TM1316	-1.4	6.6	hypothetical protein
TM1458	-1.3	6	ribosomal protein L21
TM1524	-1.8	22.4	endoglucanase
TM1525	-1.5	18.8	endoglucanase
TM1683	-1.7	31.5	cold shock protein
TM1765	-1	5.2	N utilization substance protein B
TM1786	-1.2	5.9	hypothetical protein
TM1803	-1.1	17.3	dnaJ-related protein
TM1810	-1	12.9	hypothetical protein
TM1839	-3.5	43	maltose ABC transporter, periplasmic maltose-binding protein
TM1878	-1.2	10.9	UDP-sugar hydrolase

CHAPTER 3:

Transcriptional analysis of co-located ORFs encoding a signaling peptide (TM0504) and tmRNA in the genome of the hyperthermophilic bacterium *Thermotoga maritima*

Clemente I. Montero, Derrick L. Lewis, Matthew R. Johnson, Shannon B. Conners, Elizabeth A. Nance and Robert M. Kelly

Department of Chemical and Biomolecular Engineering
North Carolina State University
Raleigh, NC 27695-7905

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ABSTRACT

Genome-wide transcriptional analysis of *T. maritima*, grown in syntrophy with the hyperthermophilic methanogen *Methanococcus jannaschii*, indicated that the DNA probe for ORF TM0504, which encodes a putative 43 amino acid peptide, was up-regulated more than 13-fold in the co-culture condition. Differential expression of this ORF corresponded to substantial, cell density-dependent, polysaccharide-based cell aggregate formation in the co-culture. Indeed, a chemically synthesized, truncated version (28-mer) of TM0504 was found to trigger exopolysaccharide formation in pure cultures of *T. maritima*. Upon closer examination of the *T. maritima* genome, it became clear that TM0504 was co-located on the opposite strand to the tmRNA (*ssrA*) gene, a hybrid molecule in bacteria implicated in a trans-translation process related to ribosome rescue. Since the microarray probe used would have detected transcription of either gene, specific DNA probes were designed and used in Real Time PCR assays to follow transcriptional response to a variety of growth conditions, including chloramphenicol challenge, co-culture with *Methanococcus jannaschii*, and as a function of growth phase in pure culture. Differential expression analysis showed that TM0504 actually varied little with respect to normal culture conditions, consistent with known quorum sensing mechanisms in which cell population density is sensed by integration of signals from community members. Transcription of the tmRNA gene, however, varied considerably, being up-regulated during conditions of chloramphenicol challenge, and down-regulated in late stationary phase where protein translation processes were minimal. Consequently, the significant up-regulation of the probe representing both TM0504 and tmRNA in exponential phase methanogenic co-culture could be attributed to a tmRNA response,

possibly arising from interactions with *M. jannaschii*. The significance of co-location of ORFs encoding a signaling peptide (TM0504) and tmRNA in *T. maritima* is not clear, although this arrangement (tmRNA with putative small peptides) was found to be common to more than 200 other bacteria for which genome sequence data are available, ranging from mesophiles (*Lactobacillus plantarum* WCFS1, *Pseudomonas syringae B378a*) to moderate thermophiles (*Chlorobium tepidum* TLS) to hyperthermophiles (*Aquifex aeolicus VF5*). Genome analysis of loci containing this construct from a representative group of bacteria showed considerable conservation with respect to the orientation of tmRNA and the putative peptides on the opposite strand. Further work is needed to explore the significance of the ubiquitous co-location of these two genes in bacterial genomes, but their possible association with two different but important microbial behaviors as seen in *T. maritima* merits closer examination.

INTRODUCTION

The bacterial "secretome" (68) encompasses a wide range of molecules residents of the bacterial periplasm or secreted to the surrounding millieu, in addition to metabolic by-products, potentially drive a variety of ecologically significant functions. Antagonistic behaviors, instigated by peptides such as bacteriocins (11, 31), can be triggered by diminishing nutritional resources or by interspecies competition (8, 35, 36, 41). On the other hand, it has also become clear that the "secretome" contains diffusible small molecules and peptides that mediate bacterial communication systems regulating population-based microbial phenomena (73). Although canonical models for bacterial signaling mechanisms have been established (19), chemically diverse molecules that act as mediators for cell-to-cell signaling continue to be discovered (73), concomitant with the expanding range of microorganisms known to be involved in inter and intraspecies communication (40, 43, 63, 73).

Recently, signaling has been connected with biologically extreme environments. For example, N-acyl homoserine lactones have been reported in cultures of the haloalkaliphilic archaeon *Natronococcus occultus* (53). Cell density-dependent, peptide-based signaling, tied to formation of polysaccharide-based cell aggregates, was recently identified in the hyperthermophilic bacterium *Thermotoga maritima* in synthrophic co-culture with the hyperthermophilic archaeon *Methanococcus jannaschii* (25, 26). Examination of genome wide transcriptional profiles indicated differential expression of a small hypothetical open reading frame, annotated as TM0504, in the *T. maritima* genome (50), in conjunction with induction of adjacent ORFs annotated as putative ABC transporter permeases and ATPases (26). An interesting feature of the 42 amino acid

peptide encoded in TM0504 was the presence of a double glycine motif located near the amino terminus of the molecule (26). This arrangement resembled leader sequences that have been identified in signaling molecules and in bacteriocins produced by Gram positive bacteria (11). These small peptides can be modified by their cognate transporters, through proteolytic release at the double glycine motif delimited N-terminal leader sequence. Indeed, when a chemically synthesized, truncated version of TM0504, excluding the putative leader sequence, was dosed into pure cultures of *T. maritima*, transcriptional activation of putative family 4 glycosyltransferases was observed along with the concomitant production of extracellular β-linked polysaccharide (26).

Subsequent re-examination of the *T. maritima* genome (50) showed that the TM0504 gene was actually co-located in the complementary strand within the tmRNA (*ssrA*) gene, which encodes a unique, highly structured RNA having both tRNA and mRNA domains. The first domain in the tmRNA molecule consists of a region that mimics a $tRNA^{Ala}$, including an acceptor stem, a T-stem-loop, and a 3'- terminal CCA that can be aminoacetylated by an alanyl t-RNA synthase (33, 70, 72). The second domain consists of an internal open reading frame (ORF), used for tagging nascent polypeptides. This unusual conformation confers on tmRNA an important biological role involving 'trans-translation' during ribosomal rescue (29). In this process, the tRNA-like portion of tmRNA, charged with alanine, acts as a surrogate tRNA to accept the nascent polypeptide. The ribosome then switches templates from the stalled mRNA to the small ORF that is used as peptide coding sequence to be incorporated into the nascent "stalled" peptide. The presence of a stop codon in the ORF included in tmRNA ensures translation termination and, hence, recycling of the ribosome (29, 79).

Current models indicate trans-translation is activated upon the endonucleolytic cleavage of ribosome-bound mRNA (23, 64), followed by the incorporation of alanylated tmRNA bound to EF-Tu, SmpB (Small protein B), and GTP into stalled ribosomes (29, 79). The role of SmpB seems to be important given the universal presence of tmRNA and SmpB in bacteria (17, 28, 79, 80). However, tmRNA is not present in *Archaea* and can be detected in *Eukarya* only as a smaller degenerated version in some plastids (16, 17). Structural analysis of available bacterial tmRNA nucleotide sequences showed a common secondary structure framework, suggesting a similar function in all bacteria. Variations were observed, however, in the number and size of the pseudoknots, supporting structures that enhance the essential functions carried out by the tRNA-like and mRNA-like domains of tmRNA (3). It is interesting that gene permutation involving tmRNA has been observed in certain bacterial groups, although essential structural features, including molecular function, are still conserved (3, 61).

located with a gene encoding a small putative peptide (see discussion below). Furthermore, it also became apparent that the probe representing this locus on the cDNA microarray, used previously to identify TM0504 (26), would not have differentiated between the peptide and tmRNA transcription. Hence, a Real Time PCR strategy was developed to separately track transcription of each of these two co-located ORFs in *T. maritima* for several environmental conditions, including methanogenic co-culture. Results from these experiments are discussed in light of possible roles of TM0504 and tmRNA in *T. maritima* as well as in other bacteria having a related construct.

MATERIALS AND METHODS

Sequence analysis of peptide-tmRNA neighborhoods in bacterial genomes. Sequence and location of the tmRNA for genome-sequenced microorganisms was retrieved from the tmRNA website (17) (http://www.indiana.edu/~tmrna/) and uploaded into Vector NTI Advance 10. (Invitrogen, Carlsbad. USA). Sequences of 200 representative bacterial genomes were selected. Identification of peptides was done using translation tables at the taxonomy browser recommended for each bacterial group at the National Center of Biotechnology Information (NCBI) and the Codon Usage Database at the Kazusa DNA Research Institute. (http://www.kazusa.or.jp/codon/) **(48).** Sequence of tRNA was retrieved from publicly available sequences at NCBI. Identification of the putative open reading frames (ORFs) was done using a lower size limit of 20 residues in all possible frames. The internal ORFs that correspond to the proteolytic tag suggested at the tmRNA website were excluded from our analysis (17).

For the identification and functional assignment of the genes co-located with the tmRNA, original gene annotations were confirmed against the COG database at NCBI (65), the Conserved Domain Database at NCBI (42), as well as using the SMART database tool for identification of functional domains (39). Five upstream and downstream genes from tmRNA were considered for each bacterium.

For analysis of tmRNA gene neighborhoods, occurrences were annotated once per functional assignment: i.e., if 10 ORFs were analyzed (5 upstream, 5 downstrean) and all belonged to the transporter/ transmembrane protein category, only one occurrence for this category would be tabulated for that bacterium. The statistical significance of the

occurrence of functional assignments was evaluated assuming independence of gene functions. Estimations were based on the discrete binomial distribution of the probability of obtaining one or more genes of that particular functional classification within 10 genes. Expected probabilities for each functional class were estimated from the known proportion of genes in that functional class in each genome, obtained from the comprehensive microbial resource available at the TIGR website (54). The difference between the expected (calculated from genome frequencies, p_e) and observed (determined from tabulating observed gene functions, p_o) proportions of the two binomial distributions being compared was tested with the null hypothesis $p_e - p_o = 0$, or no difference between the the proportions. This t-test was performed using Statlets (18) and p-values were reported for each functional category.

Growth conditions and RNA extractions. *Thermotoga maritima MSB8* was grown anaerobically on Sea Salts Medium (SSM) or BSMII media, as previously described (26, 56). Media was adjusted to pH 6.8 with NaOH (Fisher Scientific, Pittsburgh, PA), and autoclaved prior to use. As a carbon source, stocks of cellobiose or maltose solutions (Sigma Chemical, St. Louis) were prepared by filter-sterilization using 0.22 μm membrane filters (Millipore, Billerica, MA) and added to the SSM media to a final concentration of 10 mM prior to inoculation. Batch cultures (50 ml) were inoculated under N_2 headspace (high purity nitrogen; National Welders, Raleigh, NC) and were grown at 80°C for appropriate intervals in oil baths. Growth was monitored by epifluorescence microscopy cell density enumeration, as previously described (20, 26). For experiments of dynamic challenge to chloramphenicol, a 16-liter Microgen

sterilizable-in-place fermenter (New Brunswick Scientific, Edinson, NJ) was used. Eight liters of media were sterilized-in-place by heating to 100°C for 20 min, then cooled at 80°C and agitated at 200 RPM. Temperature control was achieved by using the sterilization mode of the fermenter. Continuous sparging with N_2 was done to maintain anaerobic conditions. The media was then reduced with 40 ml of 10% solution of sodium sulfide. A 2% inoculum was introduced anaerobically through positive pressurization of culture bottles directly connected with Norprene A60F tubing (Saint-Gobain, Bridgewater, NJ) to the inoculation port of the fermenter.

A similar approach was used for retrieving samples for steady state, continuous culture conditions (46). For all samples, 350 ml of culture were harvested into centrifuge bottles and cooled rapidly to 0°C by immersion in ice water followed by immediate centrifugation of the cells at 10,000 x g for 15 min at 4°C. RNA extraction was then performed following protocols previously described (15). For details of all conditions tested and experimental procedures in *T. maritima*, see Table 3.1 and references therein.

Real-Time PCR protocols. For Real-Time-PCR experiments, a gene-specific strategy was used involving a strand tag for proper identification of transcripts from the overlapping genes tmRNA and TM0504 (see Figure 3.1). All RNA samples used were pre-treated with DNAse I (Amplification grade) (Invitrogen, Carlsbad, CA), following manufacturer's recommendations. The concentration and purity of RNA samples were carefully controlled by visual inspection of denaturing agarose-formaldehyde electrophoresis gels and standard spectrophotometric measurements. For cDNA generation and Real-Time-PCR, a two-step, gene-specific strategy was adopted.

Superscript III (Invitrogen, Carlsbad CA) was used for the generation of cDNA from 2 μg of RNA sample, following the manufacturer's protocols. The generation of cDNA was performed using a single 40-mer primer per gene. The PAGE purified 40-mers were specifically designed with a 3' end complementary to the sequence of either the tmRNA or TM0504, respectively. Both primers incorporated an "alien" tag sequence of 20 bp (AGAATCCAACCGACCTCTCG) on the 5' end, not present in the *T. maritima* genome sequence. A strand-specific primer was designed to be complementary to the cDNA generated, and a primer with the "alien" tag sequence was paired to perform the PCR reaction. For details of the primers used for these experiments see Table 3.2. Real-Time-PCR reactions were performed at optimized annealing temperatures using the SYBRGREEN Supermix kit and iCycler iQ real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer's protocols. Annealing temperatures were chosen based on the quality of the PCR reaction, as monitored through the use of a melting curve analysis to ensure no non-specific amplification. All assays were performed in triplicates; reactions without reverse transcriptase were utilized as a negative control. Numerical estimates of differential gene expression were calculated with vendor-provided software (Bio-Rad Laboratories). The threshold cycle (Ct) is defined as the point at which an increase in the signal associated with an exponential growth of PCR product is detected. This phase provides the most useful information about the reaction: the slope associated with it is a reflection of the amplification efficiency. This efficiency value is used to estimate the fold changes using the formula: Fold Change = [(100 + % efficiency) / 100] $^{(Ct\ control-\ Ct\ test)}$ (55). All primers were purchased from IDT-DNA (Coralville, IA).

RESULTS and DISCUSSION

Differential transcription of tmRNA and TM0504 in *T. maritima.* The fact that a chemically synthesized, truncated version of the peptide encoded in TM0504 triggered exopolysaccharide production in *T. maritima* suggested that this peptide plays a role in the molecular ecology of this bacterium (26). Following initial work focusing on the differential expression of the peptide encoded in TM0504, it became apparent that TM0504 was located in reverse orientation and complementary to the tmRNA gene in the *T. maritima* genome, as shown in Figure 3.2A (50). The unusual organization of this genetic locus raised questions about the transcriptional regulation of tmRNA and TM0504, in addition to the physiological significance of the products of this genetic construct. The PCR probe-based microarray platform initially used for the identification of the role of TM0504 (26) included portions of the coding and template strands of both the peptide and tmRNA. Therefore, transcripts for either tmRNA or TM0504 could have hybridized to this probe, such that transcriptional response to specific conditions would have been confounded (see Figure 3.2A). In order to resolve the specific transcription of these genes, a strategy was developed in which Real-Time-PCR was adapted to independently estimate the transcript levels of the overlapping genes. This approach was then used to interrogate RNA samples taken from several conditions for *T. maritima*, including different growth phases in pure culture, chloramphenicol challenge at 100 μg/ml in chemostat culture, as well as re-interrogation of samples from syntrophic co-culture with *M. jannaschii* (25, 26, 46, 58) (see Table 3.2).

Effect of growth phase on the regulation of TM0504 and tmRNA in *T. maritima*. Differential expression of the tmRNA and TM0504 transcripts was not noted in the transition between mid-log and early stationary phase, but were observed 12 hours after onset of stationary phase (Table 3.1). The transcriptional response of tmRNA in other bacteria has not been reported, except that tmRNA levels increased in *Synechocystis* sp. before the onset of stationary phase (75). The down-regulation observed on the tmRNA and TM0504 could not be associated with the up-regulation of any particular ribonuclease. However, a statistically significant change (1.6-1.9 fold) of the transcripts of two sigma factors RpoD (TM1451) and RpoE (TM1598) was observed, which might be related to down-regulation of most "house-keeping" genes linked to late stationary phase.

Regulation of TM0504 and tmRNA in *T. maritima* during chloramphenicol challenge. Translational inhibiting antibiotics, such as kanamycin, gentamycin (1) and chloramphenicol (66), cause read-through of stop codons and increase the frequency of ribosome stalling (66). These effects presumably invoke action by tmRNA, although this has not been reported. In *B. subtilis*, up-regulation of the tmRNA transcript was observed under several stress conditions, including heat shock, and exposure to ethanol or cadmium chloride (47). In some bacteria, tmRNA has been associated primarily with ribosomal rescue, without necessarily enacting proteolytic degradation of partially translated peptides (21). Here, upon challenge to the translational inhibitor chloramphenicol (100 μg/ml), *T. maritima* growing in continuous culture demonstrated significant up-regulation of the tmRNA gene (+40-fold at 5 min after dosing vs. before

challenge), presumably reflecting the deleterious effect of the antibiotic on translational processes (Table 3.1). Little or no change was noted for TM0504. At this point, it is unclear how tmRNA is regulated in *T. maritima*, but it is noteworthy that during chloramphenicol challenge, there was up-regulation of genes associated with the stringent response (5, 46). These included a pppGpp synthase/hydrolase RelA/SpoT (TM0729), and GppA (TM0195), a putative guanosine pentaphosphate, exopolyphosphatase, all probably associated with turnover of pppGpp and the second messenger ppGpp (5, 37, 38). It has been shown in *E. coli* that ppGpp inhibits the expression of toxin/antitoxin stress response endonucleases that are associated with the degradation of tmRNA and the bactericidal effect of chloramphenicol (7, 60).

Differential expression of co-located TM0504 and tmRNA genes in *T. maritima* co-culture with *M. jannaschii*. Previously, TM0504 was identified as among the most highly up-regulated genes (13-fold) in *T. maritima* grown to high cell density in co-culture with the methanogen *Methanococcus jannaschii* (26). Re-interrogation of samples from that experiment here using Real-Time-PCR revealed that the 13-fold increase in the co-culture was primarily related to the up-regulation of the tmRNA gene. Table 3.2 shows that the tmRNA gene showed an 8-fold increase relative to the pure culture, while the TM0504 gene was significantly down-regulated for this same comparison (over 30-fold). The reasons for up-regulation of tmRNA were not clear but this may be related to the production of antimicrobials affecting *T. maritima* by the co-culture partner *M. jannaschii*. Here, down-regulation of several ribosomal proteins (TM1470-TM1475, TM1478-TM1505, TM1566, TM1590-TM1592) was noted, as were

components of the ATP synthase (TM1608-TM1614). The synthesis of amino acids leucine, isoleucine and valine seemed to be also down-regulated (TM1243-TM1245, TM1248-TM1251). Up-regulation of TM0729 (an SpoT/RelA homolog) and some genes belonging to the COG category of defense mechanisms, including two efflux pumps (TM1701, TM0815) that belongs to the Mat E family of multidrug/Na^+antiporters (22), was observed. In addition, genes associated with detoxification functions such as TM0395 (Ferredoxin oxidase), TM0396 (Iron sulfur cluster oxidase) and RNAse P (*rnpB*) was also significantly up-regulated in the co-culture (+7.7-fold) (26). RNAse P is associated with the processing of tmRNA (4). The reasons behind this are unclear but decay of this molecule has been linked directly to the stringent response (6). The concerted up-regulation of *rnpB* with TM0729, which might have pppGpp hydrolase activity, may be indicative of a condition that does not trigger a stringent response (6). The reason for significant down-regulation of TM0504 in the co-culture aggregate compared to pure culture was not clear but may relate to the fact that cells no longer participate in population-based behaviors (i.e., exopolysaccharide formation) once they are constrained to aggregates. There is also increasing evidence suggesting that the cell-to-cell signaling mediated by the quorum sensing system is strongly affected by environmental factors other than the cell density, for example, by concentration of nutrients (78) or concentration of iron and oxygen levels in aerobic cultures (30).

Identification of small ORFs encoded within the tmRNA gene locus in bacteria. As shown on Figure 3.2.A, the TM0504 ORF encodes a peptide situated in the complementary strand of tmRNA. Whether this arrangement is common to other tmRNA

genes was examined for all reported genome-sequenced bacteria. Given that tmRNA is a highly conserved molecule with a relatively high GC content, a lower frequency of start and stop codons was expected. However, small ORFs could be identified on the opposite strand of the tmRNA molecule in all bacterial genomes. Indeed, small ORFs associated with "conventional" tRNAs were also found. Despite the fact that tRNA genes are on average about half of the length of tmRNA, putative ORFs were detected in 49%, 38%, 28%, and 33% of the tRNAs in *Lactobacillus acidophilus*, *Escherichia coli*, *Lactococcus lactis*, and *T. maritima*, respectively. As a consequence, this approach will identify many irrelevant ORFs. Therefore, analysis was restricted to ORFs similar to the location associated with TM0504, which is encoded in the 3' end "anti-sense" strand of the tmRNA gene, and to ORFs located on the 5' end on the complementary strand of tmRNAs that had a circular gene permutation, commonly called two-piece tmRNAs (61).

For the 200 tmRNA genes analyzed, 26 corresponded to two-piece tmRNAs (3, 61), while 155 had a 3' end complementary strand encoding a small ORF. From this group, 127 had a conformation similar to that observed in TM0504 (Figure 3.2.A) in which the ORF starts exactly two bases after the 3' end of the tmRNA (Figure 3.2.B); the size range of these peptides varied from 20 to 107 with most having less than 50 amino acids in the putative encoded peptide (Figure 3.3). It is noteworthy that an annotated assignment of these putative peptides is not typically reported for the 200 bacterial genome sequences examined. In fact, in only 3 other cases, in addition to *T. maritima*, was the TM0504-related peptide annotated as a hypothetical protein: LP2510 in *Lactobacillus plantarum WCFS1* (32), TC0287 in *Chlamydia muridarum Nigg* (57), and CpB0109 in *Chlamydia pneumoniae TW-183* (unpublished).

Analysis of tmRNA gene neighborhoods for selected bacteria. In the case of *T. maritima*, TM0504 is co-located with an ABC transporter, suggesting an export pathway for the signaling peptide. Genes adjacent to tmRNA in *T. maritima* and other selected bacteria were examined in order to elucidate possible functions that might be associated with putative peptides. As can be seen in Figure 3.4, many of the associated ORFs (± 5 ORFs from the tmRNA/peptide locus) are related to SmpB (as might be expected) but there is also a high proportion of transport and transmembrane proteins, with high statistical significance (see Table 3.3), based on analysis of the average proportion of functional categories within genomes available at TIGR and the Comprehensive Microbial Resource (54). Features of the tmRNA-peptide locus are reviewed here for selected bacteria in comparison with *T. maritima* (see Figure 3.5) shows the alignment of peptides, seems clear that there are similarities among the amino-terminal end of these molecules, it is also interesting the conservation of leucine or valine in the carboxy-terminal end of these peptides.

Thermotoga maritima MSB8. In previous studies, it was noted that the strong up-regulation of the TM0504 ORF occurred when *T. maritima* was grown to high cell densities in methanogenic co-culture (26). Figure 3.6 shows the gene neighborhood containing TM0504 and tmRNA. TM0503 is annotated as a transmembrane component of a “dipeptide transporter system”, TM0502 is also a permease for such systems, while the accompanying TM0500 and TM0501 are putative ABC transporter ATPases (see Figure 3.6). This transporter is apparently not associated with carbohydrate utilization (9) and may be involved in exporting TM0504 (26)

***Aquifex aeolicus* VF5.** *A. aeolicus* is a deeply branched, non-spore-forming, gram negative bacteria, initially isolated from the Aeolic Islands in Italy (10). It is one of the most hyperthermophilic bacteria known, capable of growth at temperatures as high as 95°C. Analysis of the genome region associated with the tmRNA present in this bacterium shows the co-location of a putative peptide encoded in the complementary strand of the tmRNA. The putative peptide consists of a pre-peptide of 60 aa (VEAAGIEPASEDGGHIRGSTGLARVQDFPSGRPRASSPRVARVRCQLLSPVRPSSPSGCL). The encoded peptide has a double glycine motif (11), in its mature form based on cleavage at this point, leads to an increase in pI of from 10.19 to 12.00. Analysis of the region associated with the peptide shows that it is positioned between apparently unrelated genes, i.e., a long stretch of ribosomal proteins AQ1641a – AQ1654, and a transposase AQ1640. However, the most significant finding is the co-location of the tmRNA TM0504 like peptide and AQ1641 (see Figure 3.6). AQ1641 is a putative family 1 glycosyltransferase related to a glycosyltransferase EpsG in *Streptococcus thermophilus*, previously implicated in the synthesis of a texturizing exopolysaccharide used in yogurt (62); note that glycosyl transferases/synthases genes were up-regulated upon TM0504 dosing in *T. maritima* (26)

Chlorobium tepidum TLS. *C. tepidum* TLS is a gram negative, rod-shaped, thermophilic, green sulfur bacterium that was initially isolated from an acidic high sulfide hot spring in New Zealand. (74). *C. tepidum* TLS grows only under anaerobic, photothropic conditions and has a unique photosynthetic machinery. Based on the recently available genome sequence (13), it was possible to identify a genome neighborhood for tmRNA that apparently has similarities to the *T. maritima* locus (26).

Co-located within the tmRNA gene is a putative peptide of 61aa (VEMAGLEPASRTLLDAVTTLISGGWFFVVRLHRQQRSSPYPDWLPAHPLGHECT-ELTCANP). This peptide also has a double glycine motif and a 22-residue leader sequence, and is encoded in the same orientation of a putative ABC transporter system (see Figure 3.7). In addition to this genomic organization, a two-component system (CT0658 and CT0657) can also be identified (not shown). In its natural environment, *C. tepidum* is usually found in consortia with chemoorganotrophic bacteria, in which interdependence leads to synchronization of cell division among the members of this association (69). In the case of this consortium, the heterotrophic bacteria are unable to grow without the phototrophic counterpart (69).

Lactobacillus plantarum WCFS1. *L. plantarum strain WCFS1* refers to a single colony picked from a plate of the human saliva isolate *L. plantarum NCBIMB 8826* (32). Its genomic sequence at 3.3Mb is the largest among all lactic acid bacteria (32). This large genome may be related to the diversity of environmental niches containing *L. plantarum*. *L. plantarum* can utilize a broad range of fermentable carbon sources and is a frequent isolate from fermentation processes of vegetables and some artisan and industrial dairy and meat fermentations. *L. plantarum* is also frequently isolated from the gastrointestinal tract of humans and animals (44). In *L. plantarum*, the putative peptide is annotated as a putative ORF (LP2510). This 80 aa peptide (MEMARVELASKHIAPRISTLIFILFKFHHCKRRMTGHPAMTNLISLFSQLQVEALS VSPLNLGPRSRTWTILGGSTLSAY) contains a GG motif that, if cleaved, changes the pI from 10.91 to 5.24. Analyses of the neighboring genes in the LP2510 neighborhood

indicates the presence of several putative transporter subunits and integral membrane proteins. These include (see Figure 3.7) LP2509, which has two PFAM DUF9 domains, usually present in drug metabolite permeases (2, 39). LP2508, annotated as a hypothetical protein containing an PFAM ACT domain, an amino-acid binding domain present in several enzymes that are regulated by the amino acid concentration (2).

Downstream of these ORFs is an uncharacterized PFAM DUF711 protein LP2507. LP2506 and LP2505 are members of a two-component regulatory system (32). Upstream of tmRNA and its putative peptide, the following are located: LP2512, an integral membrane protein with 5 transmembrane domains; LP2513, a small hypothetical protein with a pI of 10.33; and a permease subunit of a putative ABC transporter LP2514.

Pseudomonas syringae B728a. *Pseudomonas syringae pv. syringae strain B728a* is a characteristic epiphytic phyto-pathogen associated with the brown spot disease on beans. The stain *B728a* is usually associated with the leaf surface and is resistant to rapid changes in temperature, low water content and solar radiation. Another distinctive feature of this pathogen is the capacity to synthesize both ice nucleation proteins and antifreeze proteins, promoting or inhibiting the formation of ice crystals on the leaf surface (14). Within the tmRNA locus (Figure 3.7), a small peptide of 20 amino acids can be identified (MEPGGFEPPSASTPLSVLHA), containing a putative GG motif. An interesting feature of this gene neighborhood is the presence, in the same orientation as the putative peptide, of a “polyketide cyclase” that might also be associated in some sort of post-transcriptional modification. It is noteworthy that PSYR4205 is encoded in the same orientation as the peptide. PSYR4205 is 41% identical and 58% similar to an insecticidal high molecular weight toxin TcdA1 (76). The mechanism of action of TcdA1 is still

unknown but it has been already cloned into plants conferring resistance to caterpillar pests at levels comparable to the *B. thuringensis* toxins (76). A transmembrane protein (PSY4206) containing a GGDEF domain is also located near PSY4205. This domain is associated with diguanylate cylase activity and also to modulation of cell surface structures via post-translational allosteric activation of proteins, such as glycosyltransferases (24). This construct may be related to the putative peptide-based signaling system, coupled with a cyclic diguanylate cyclase signal transduction mechanism, proposed for TM0504 in *T. maritima* (26). The co-location of components of these systems in *P. syringeae* might be more than a coincidence. The putative peptide-based system in *Pseudomonas syringeae B728a* may be triggered by exposure to unorthodox environmental stimuli, such as caterpillar gut.

Co-localization of tmRNA with small peptides. The postulate that a biologically-active peptide is encoded and independently transcribed in the strand opposite tmRNA is intriguing given the rarity in which ORF co-location occurs in bacteria. In *T. maritima,* we have presented evidence that tmRNA and TM0504 are transcribed independently under a variety of growth conditions, and that within this sole sequence exists that capability for both ecological interactions and ribosome rescue. While this phenomena has yet to be described in other microorganisms, a previous study utilizing Northern Analysis suggested that transcription of an ORF corresponding to a putative small peptide co-located with the tmRNA occurred in *Bradyrrhizobium japonicum* (12). This result was interpreted to be due to contamination of the RNA sample, given that the bacteria were cultivated with plant tissue. Upstream of 5’ end of

tmRNA and in the same orientation of the putative peptide in *B. japonicum*, there are two transmembrane proteins. These proteins (B116517 and B116516) have sequences similarity to chromate transporters, which is striking given that transporters are located upstream from the putative TM0504 peptide in *T. maritima*. Efforts to locate the translated peptide in *B. japonicum* were unsuccessful, but this putative peptide is also acationic and has a GG motif. In *Salmonella enterica* serovar *typhimurium*, insertion mutagenesis of the 3' on the tmRNA molecule has been implicated in reduction of virulence and inhibition of expression of genes including a toxin-related (cvpA) gene; the interpretation of these results is confounded by the insertion of a prophage in the *ssrA* region. This observation was attributed to a regulatory role of tmRNA in which alteration of DNA interactions or translation associated to this gene directly affect virulence (27). Analysis of the gene neighborhood among the bacterial strains selected here indicates that hypothetical proteins are among the most frequent genes found, but the high frequency, in which transporters/transmembrane proteins, proteases and peptidases are encoded, that opens the possibility of similar signaling systems to the one previously described for *T. maritima* (26). In *Enterobacteriacea*, it was previously reported that the benefit of the juxtaposition of SmpB and tmRNA is minimal (77). This seems to be the case, even using less restrictive screening criteria, and analyzing 5 ORFs upstream and downstream of the tmRNA showed only 63 genomes exhibited this feature. Apparently, based on currently available information, the co-location of tmRNA and SmpB seems to be most frequent in the Phyllum *firmicutes*.

overhang observed in TM0504, suggesting an important function. Limitations on the t-

acceptor loop and in the mechanism of processing of tmRNA are the most probable explanations. But, this may also imply similar mechanisms of processing *in vivo.* 3' tmRNA processing by itself remains undefined, but apparently requires RNAse R, RNase III, RNAse E and P (4)

Conclusions. The co-location and independent regulation of an unusual locus of overlapping genes in the tmRNA gene neighborhood in *T. maritima* has been identified. Steric limitations likely limit both transcripts being read simultaneously within the 400 bp region but this has not been examined. Examples of regulation of overlapping genes in bacteria are relatively rare. However, one example is the pic/set locus in *Shigella*, corresponding to a mucinase and enterotoxin arrangement that seems to be differentially transcribed (46) and also the RepEA and RepEB that show differential transcription during the T4 bacteriophage DNA replication (71). Overlapping genes may be a way compress genome size in species subject to reductive evolution (59). *T. maritima* is a free-living bacterium that would seem to have frequent opportunity to expand its genome through exchange of genetic elements with co-located microorganisms, including the archaeal hyperthermophiles (45, 50-52). This feature of overlapping genes at the tmRNA locus might in fact be primitive. Certainly, an interesting arrangement was noted in *A. aeolicus*. It is also possible that this feature has been conserved across several bacterial phyla as a possible means for protecting against mutation events (34, 59). In the case of the overlapping peptide/tmRNA, the constraints associated by a functional t-alanyl-acceptor region imply a locus restrictive for modifications, which based on the alignments provided at the tmRNA database, seems to be the case (80). More work is

needed to determine the significance of this gene co-location, including testing for evidence that specific microbial behaviors arise through peptide-induced regulation.

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Table 3.1. Differential expression of ORFs encoding TM0504 peptide and tmRNA.

	TM0504		tmRNA	
GROWTH PHASE EFFECT (25)				
Pure Culture – Fermenter/Sparging	**Ct***	**Fold**	**Ct***	**Fold**
Mid-log (ML)	30.0 ± 0.2		18.6 ± 0.1	
Early stationary (ES)	30.1 ± 0.2		18.5 ± 0.1	
Late stationary (LS)	32.8 ± 0.1		30.3 ± 0.1	
ES vs. ML		**1.0**		**1.0**
LS vs. ML		**-5.4**		**< -100**
Co-Culture – Fermenter/Sparging				
Mid-log (ML)	30.8 ± 0.3		19.1 ± 0.1	
Early stationary (ES)	30.3 ± 0.2		18.8 ± 0.1	
Late stationary (LS)	32.9 ± 0.3		30.2 ± 0.2	
ES vs. ML		**1.0**		**1.0**
LS vs. ML		**-3.5**		**< -100**
ANTIBIOTIC EFFECT (46)				
	Ct*	**Fold**	**Ct***	**Fold**
Chemostat before CAM challenge	29.1 ± 0.4		28.4 ± 0.3	
Chemostat 5 min after CAM challenge	28.5 ± 1.0		22.9 ± 0.1	
Chemostat 30 min after CAM challenge	28.0 ± 0.9		23.8 ± 0.2	
CAM challenge: 5 min vs. before		**1.0**		**40.3**
CAM challenge: 30 min after vs. before		**2.0**		**22.8**
CO-CULTURE EFFECT (26)				
Serum bottles – No sparging	**Ct***	**Fold**	**Ct***	**Fold**
Pure culture (mid-log)	30.3 ± 0.4		21.6 ± 0.04	
Co-culture (mid-log)	35.5 ± 0.1		18.7 ± 0.04	
Co-culture vs. Pure culture		**-30.6**		**8.0**

* = Threshold cycle (55)

Fold Change = [(100 + % efficiency) / 100] $^{(Ct\ control - Ct\ test)}$ (55)

Table 3.2. Primers used for Real-Time-PCR probes of overlapping ORFs encoding TM0504 peptide and tmRNA in *T. maritima.*

Target	Name	Feature	Sequence*
TM0504	40TA	40-mer + TAG	5’AGAATCCAACCGACCTCTCGGAAGG CTGTGGGAGAGGACAC 3'
	sTA	Sense primer	5' - ATTCGAACCCCCGTCCGAAG - 3'
tmRNA	40TT	40-mer + TAG	5'AGAATCCAACCGACCTCTCGTGAGTT TTCCCGATTCAGGC - 3'
	sTT	Sense primer	5' - GGTTCGACGGGGATGGA - 3'
TAG	TAG	Anti-sense primer	5’-AGAATCCAACCGACCTCTCG-3’

* Underlined sequence represents the section of the TAG for strand-specific Real-Time-PCR.

Table 3.3. Statistical significance of the functional assignment of genes adjacent to tmRNA					
Role Category	**% Genome**	**Expected proportion (Pe)**	**Observed proportion (Po)**	**p-value**	**Comment**
SmpB	0.01	0.09	0.32	3.58E-09	Higher*
Mobile Elements, Phages	0.03	0.23	0.31	7.16E-02	Higher
DNA metabolism	0.04	0.35	0.49	4.56E-03	Higher*
Protein Synthesis	0.06	0.48	0.23	1.75E-07	Lower*
Nucleotide and AA synthesis	0.05	0.39	0.30	9.13E-02	Lower
Hypothetical proteins	0.43	1.00	0.85	2.47E-07	Lower*
Cellular Processes	0.05	0.43	0.17	1.40E-08	Lower*
Regulatory proteins and Signal transduction	0.06	0.48	0.10	0.00E+00	Lower*
Protein Fate	0.04	0.35	0.41	2.16E-01	Higher
Transporter and Binding proteins	0.09	0.63	0.91	2.88E-11	Higher*
Energy metabolism and Cell envelope	0.18	0.86	0.25	0.00E+00	Lower*
Other	0.08	0.58	0.22	2.02E-13	Lower*

(*) Statistically significant
% Genome: Refers to the average % of genes classified in a specific category among the genomes analyzed (54)
Expected proportion: Value of $P(X \geq 0)$ Binomial distribution
Observed proportion: Empirically estimated occurrence.
p-Value= Statistical significance.
Higher or lower values represent the respective comparison between the expected value and the observed proportion in genomes in which the functional category was annotated.

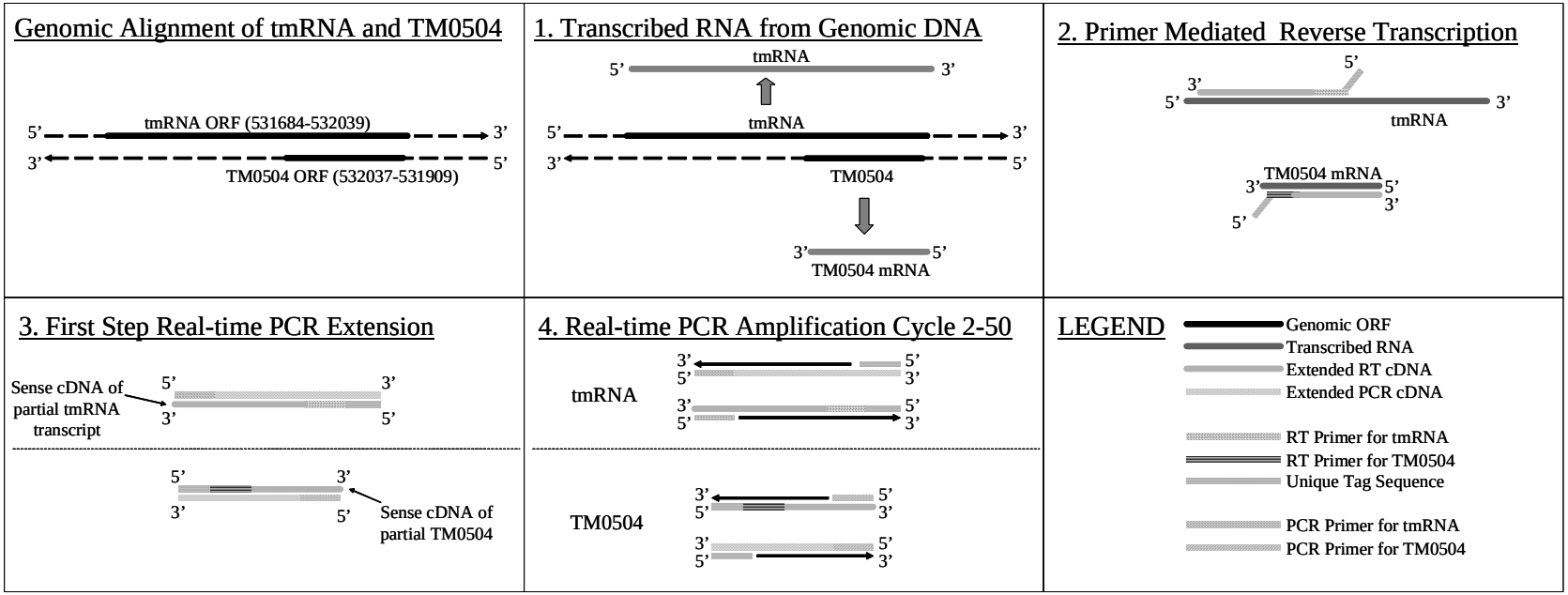

Figure 3.1. Strategy of Real-Time-PCR detection of transcripts from overlapping genes: Transcripts were identified from overlapping genes through the use of a two step Real-Time-PCR protocol in which primers with specific tags allowed for the independent identification of each strand

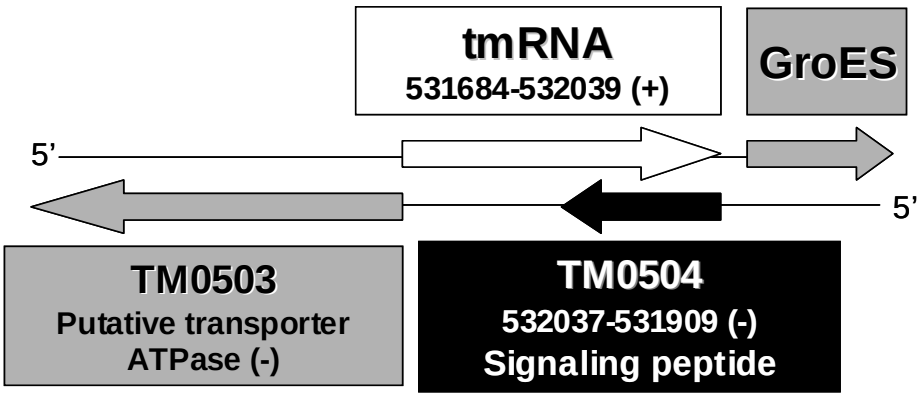

Figure 3.2A. Co-location of TM0504 and tmRNA genes in *T. maritima* genome.

Figure 3.2.B. Characteristics of the 3' end of the TM0504. TM0504 is located in the 3' end, t-RNA like domain of the tmRNA. In *Thermotoga maritima* TM0504 is encoded after two bases complementary to the terminal CCA sequence. This CCA sequence in tmRNA is associated with the aminoacylation of the molecule.

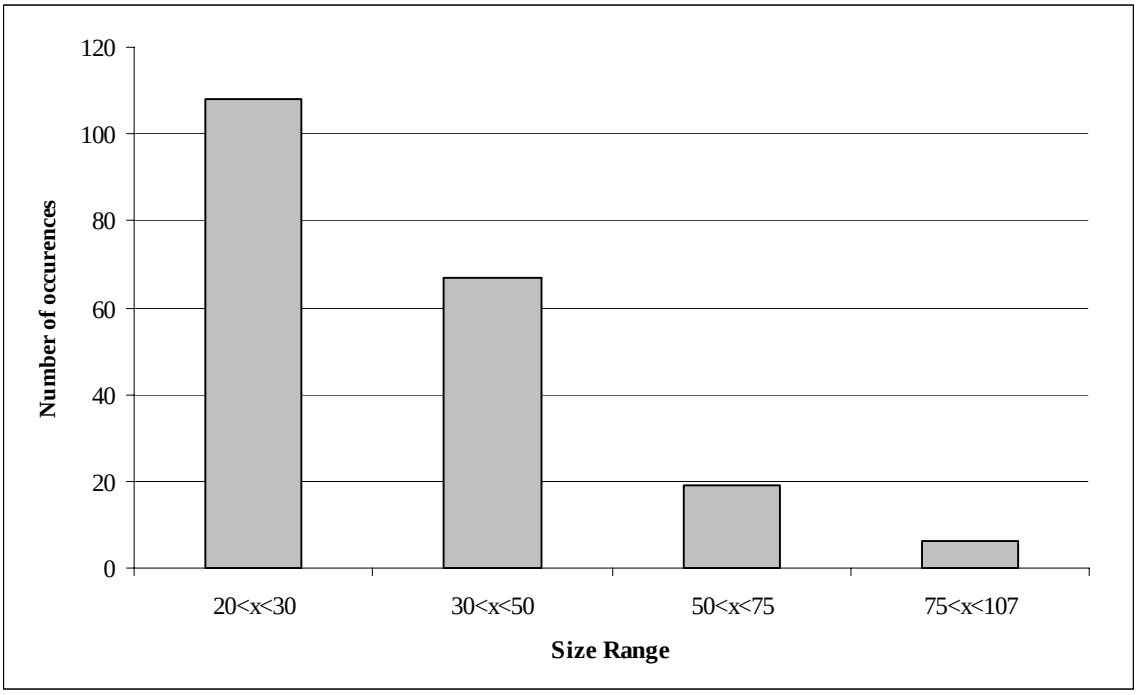

Figure 3.3. Size distribution among putative TM0504-like peptides in 200 selected bacterial genome sequences from TIGR and NCBI (49, 67) The occurrence of small peptides complementary to the tmRNA locus is more frequent than all the other size ranges combined, the TM0504 like tmRNA (107 aa) is for *Porphyromonas gingivalis*. *T. maritima* TM0504 is 42 residues long.

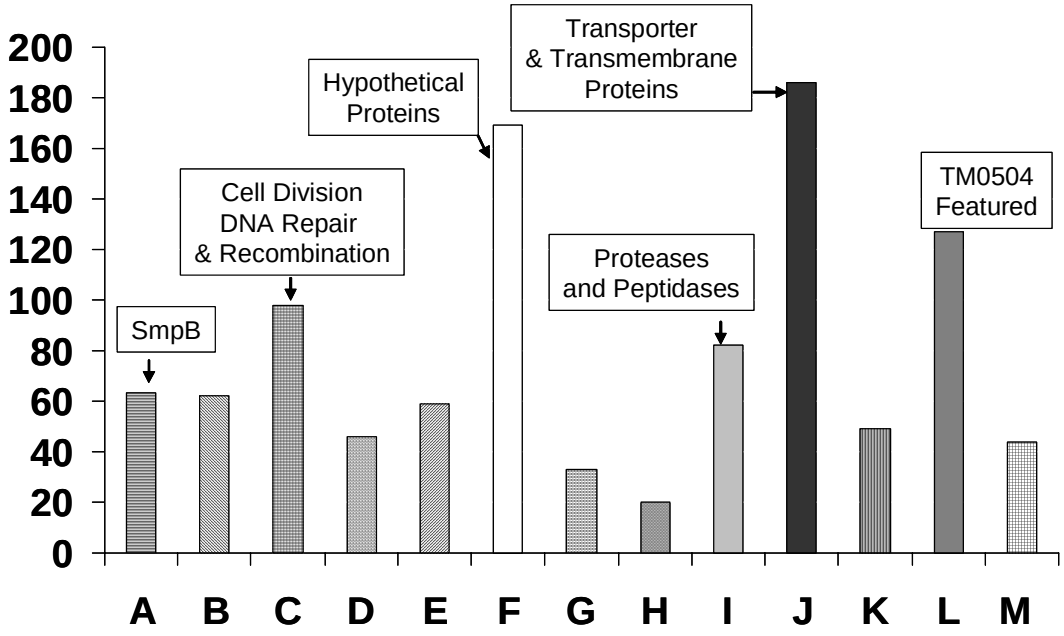

Figure 3.4. Functional assignment of the genes adjacent to tmRNA in 200 selected bacteria. Functional assignment of genes co-located with tmRNA, was tabulated as explained in materials and methods, briefly 5 genes upstream and downstream of the tmRNA were considered and its occurrence as a single event per functional category. Legend: A= SmpB, B=Cellular proceses/Cell division, C= DNA metabolism (Cell division DNA repair and recombination), D= Protein synthesis, E=Aminoacids and Nucleotide synthesis. F= Hypothetical proteins, DUF family proteins, G=Cellular processes H= Two component systems and Regulatory proteins, I= Protein Fate (proteases and peptidases), J= Transporters and transmembrane proteins, K= Energy metabolism and cell envelope, L= TM0504 featured corresponds to TM0504 like peptides with similar features at the Start codon, as observed in *T. maritima*, M= Other functions: Stress and intermediary metabolism. For statistical significance of these functional assignments please see Table 3.3.

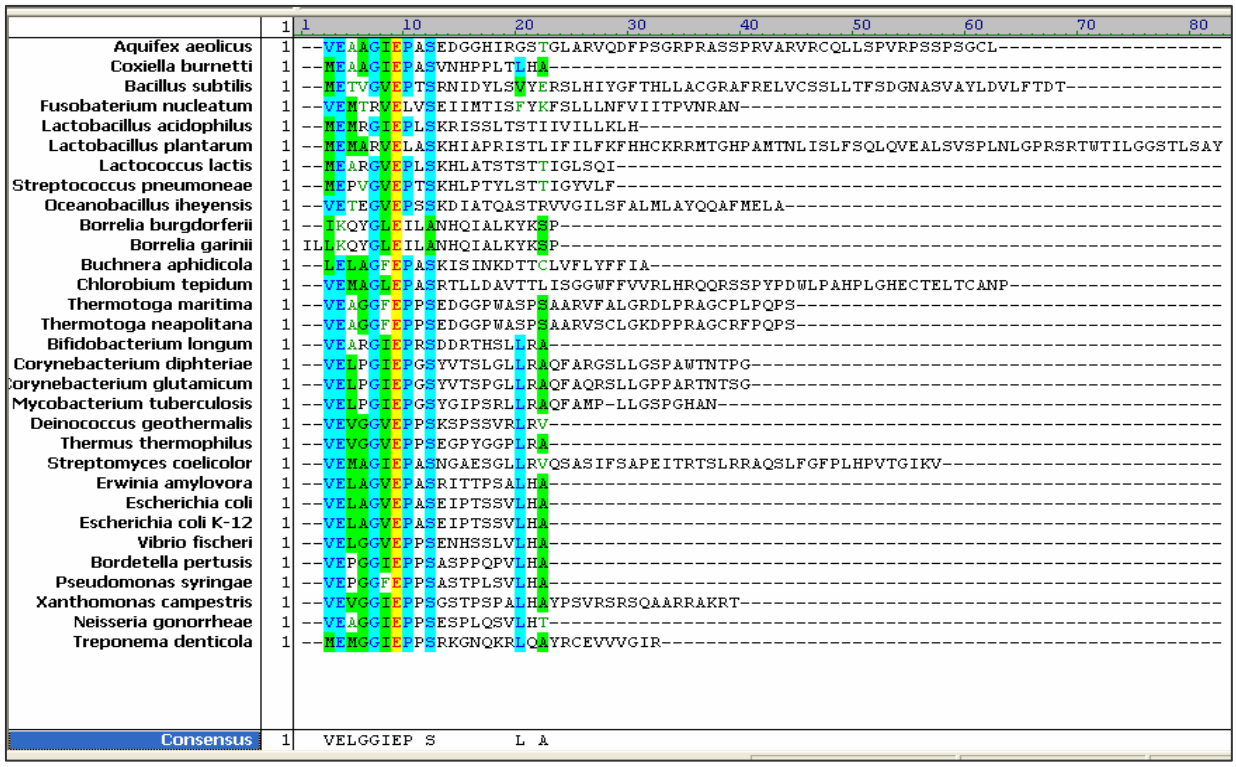

Figure 3.5. Alignment of selected TM0504-like peptides. Alignments were performed using the Align X/Clustal W tool of vector NTI. As shown, the amino terminal end of the putative peptides is conserved among phylogenetically diverse groups of bacteria. The carboxy-terminal end is distinct for every phylogenetic group. While the amino-terminal end might be linked to sequence restrictions in the alanyl acylation site of tmRNA.

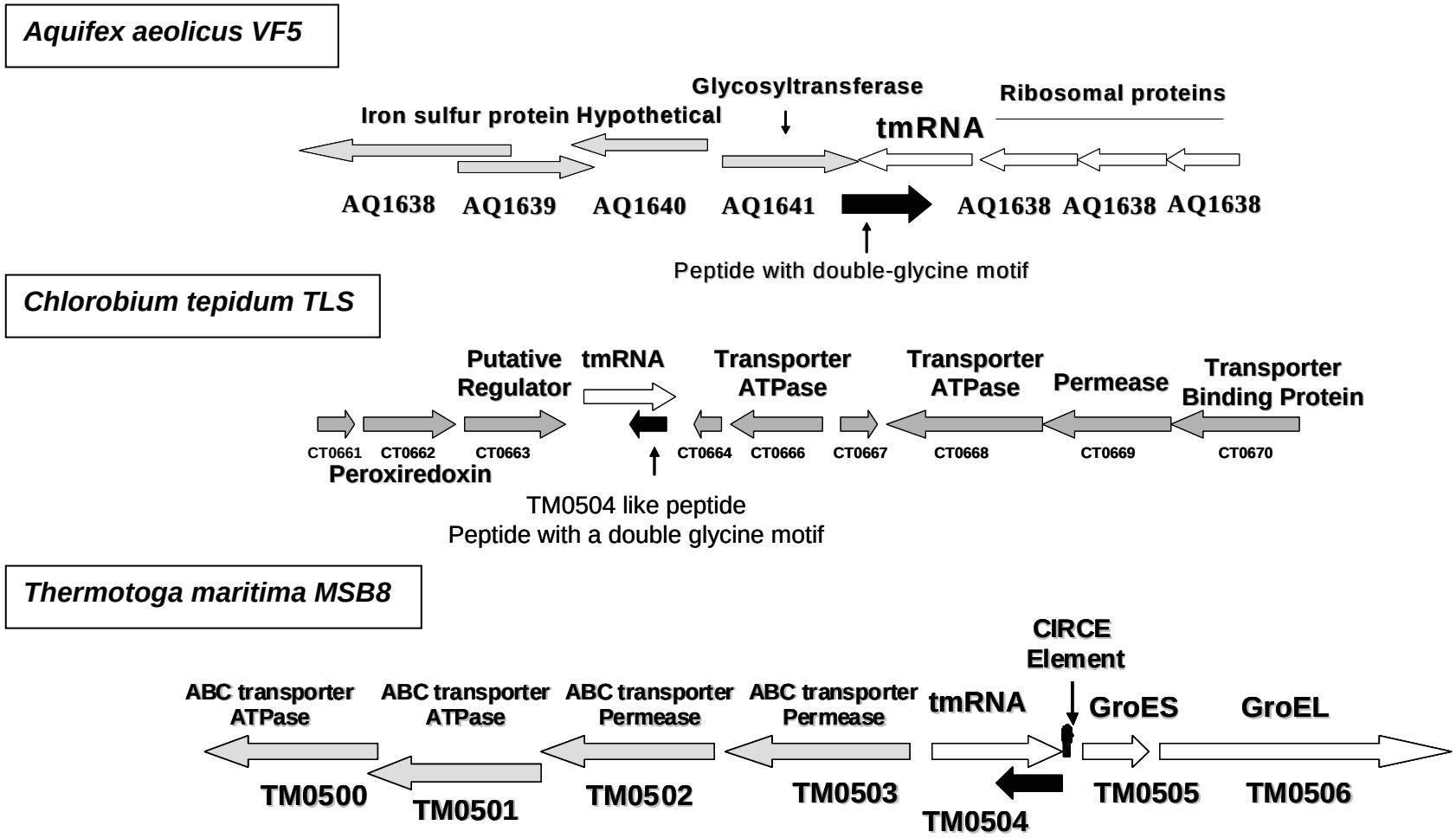

Figure 3.6. Genome organization of the tmRNA locus in thermophilic and hyperthermophilic bacteria: *Aquifex aeolicus VF5*, *Chlorobium tepidum TLS* and *Thermotoga maritima MSB8.*

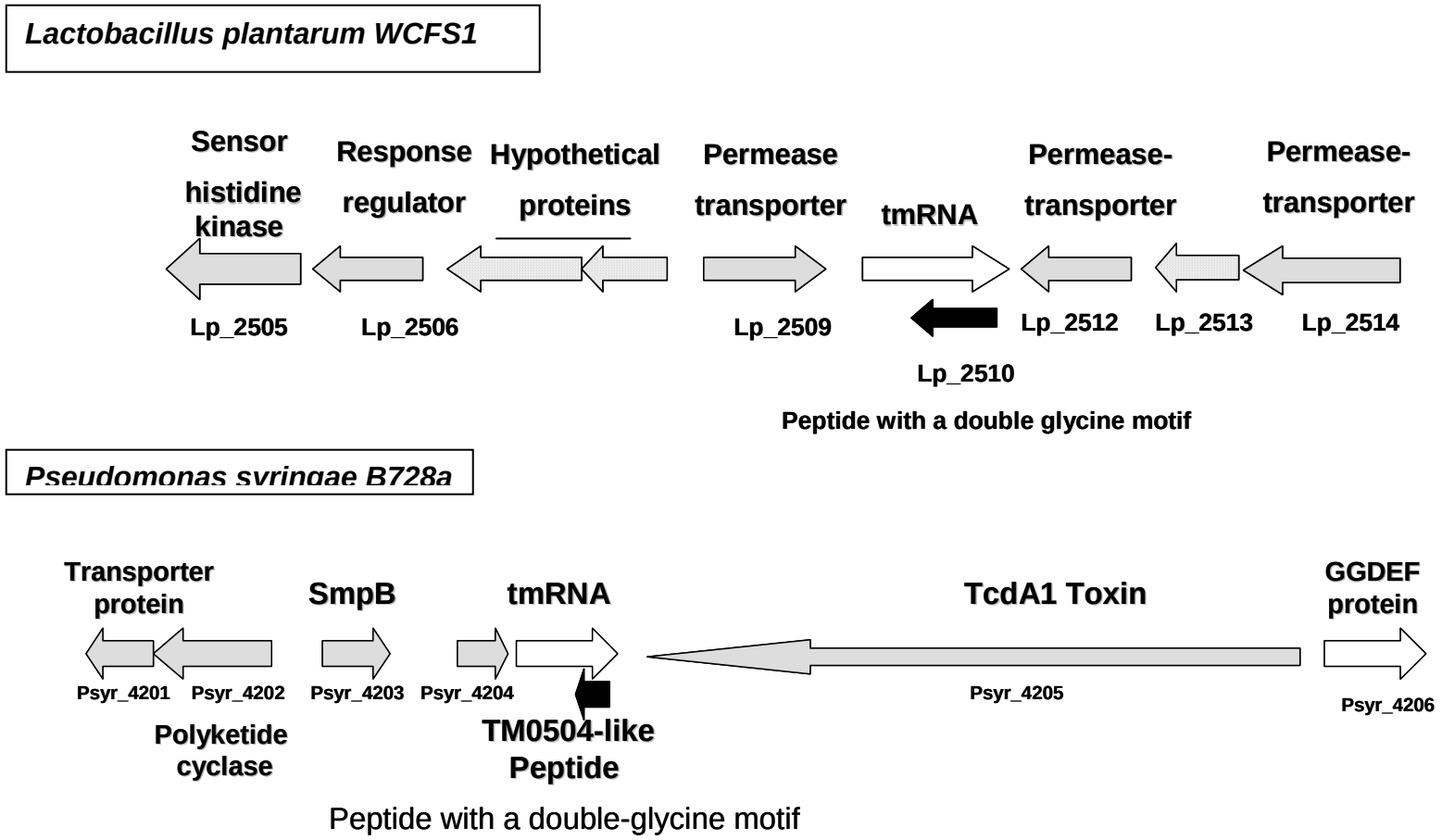

Figure 3.7. Genome organization of the tmRNA locus in representative mesophilic bacteria: *Lactobacillus plantarum WCFS1* and *Pseudomonas syringae B728a*

APPENDIX A:

Analysis of tmRNA gene neighborhoods for selected bacteria

Analysis of tmRNA gene neighborhoods for selected bacteria

The present section describes the gene organization adjacent to the tmRNA in selected bacteria. It illustrates the diversity of functional roles that can be assigned to these genes based on their annotation and by the use of bioinformatics tools such as the COG database at NCBI (64), the Conserved Domain Database at NCBI (42), and the SMART database tool for identification of functional domains (39).

***Acinetobacter sp.* ADP1.** A ubiquitous and versatile anaerobic saprophyte capable of natural competence, *Acinetobacter* is also an important opportunistic nosocomial pathogen (25). The *Acinetobacter* sp. strain ADP1 sequence was obtained from a highly competent soil isolate. The analysis of the tmRNA regions reveals that on the same orientation of the putative TM0504 like peptide a transmembrane protein (domain DoxX) is co-located ACID0931 (see Figure A.1) (3, 20). However, the role of this group of proteins is not known, but similar proteins have been identified in a quinol oxidase of *Acidianus ambivalens* (32). Positioned with the same orientation, a glycerol kinase can be identified which might be involved in the glycolipid metabolism. It is intriguing, however, that a gamma-glutamyl transpeptidase (GGT) is located nearby; this enzyme belongs to the family of bacterial and mammalian gamma glutamyl transpeptidases (E.C. 2.3.2.2) that catalyze, among other things, the reaction of 5-L-glutamyl-peptide with an amino acid to form a peptide and 5-L-glutamyl amino acid, according to the description of PFAM PF01019. This family also has homology to cephalosporin acylases.

The *Acinetobacter* GGT protein has homology of 45% identity and 59% similarity to the GGT-1 from *E. coli*. It is interesting that the peptide found on

Acinetobacter VEMABVEPASASTTLENTTCLDIVYCFNTQ ends in a glutamine residue, which suggests that is a potential substrate for posttranslational modification.. In this region (see Figure A.1.) homologs of VceB and VceA (ACIAD0926, ACIAD0927) can be identified. VceB and VceA are an inner membrane proton anti-porter and a periplasmic adaptor proteins characterized in *Vibrio cholerae* which are associated with multi-drug resistance in *E. coli* and other bacteria (9). However the potential role of this transporters in the exclusion of putative signal molecules needs to investigated experimentally.

Bacillus thuringiensis serovar konkukian 97-27. *B. thuringiensis* is a gram-positive bacterium usually associated with soil samples. One of the most important properties of this group is the synthesis of toxins that are routinely used for the control of several groups of insects (33). The implementation of this strategy results in sales of over $90M per year, which represents 2% of the worldwide sales of the agrochemical sector (18). In particular, the genomic sequence available of *B. thuringiensis serovar konkukian* 97-27 belongs to a clinical isolate from an infected wound of a patient with septicemia (10). Analysis of the tmRNA gene shows the presence of a several putative TM0504 like peptides. It is difficult, however, to identify peptides with relevant biological activity. The presence of a nearby ORF BT9727_4805 that corresponds to a member of the CAAX family, cysteine amino terminal proteases (see Figure A.1), may indicate that the relevant substrate contains at least one Cys residue. Among all the possible ORFs encoded within the tmRNA, there is only one that possesses cysteines (MEGGTFSAIKQLKKDCFVCQL). Most of the CAAX proteases characterized to date belong to eukaryotic organisms and have an endoproteolytic/regulatory function.

However, similar proteins have been found in operons associated with the synthesis of bacteriocins, such as plantaricin A. (6). It has been suggested that these CAAX proteases play a role in the maturation and transport of bacteriocins or in providing some sort of mechanism of resistance to them (29). Downstream to this section, there is a set of genes, including smpB and a ribonuclease (vacB), that might to be directly associated with the role of tmRNA during trans-translation or in its transcriptional regulation (39)**.** Therefore this genomic organization present some features that should be considered to the study of such genes in relationship of this bi-functional genetic locus.

***Enteroccocus faecalis* V583.** *E. faecalis* is an opportunistic pathogen that causes urinary tract infections, bacteremia, and infective endocarditis. It is responsible for emergent diseases associated with nosocomial infections mediated by multi-drug resistance strains. *E. faecalis* V583 is a vancomycin-resistant strain isolated from a chronically infected patient. Its genomic sequence became available in 2002 (37). Several putative peptides were encoded within the tmRNA gene but none has the extreme 3'end localization similar to TM0504 on *T. maritima*. However, a 37 cationic residue (pI 11.3) could be identified within the tmRNA's complementary strand. (see Figure A.2). In this gene neighborhood it is interesting the identification of Ace (24), a collagen-binding protein previously identified as a virulence factor that is associated with the binding of *E. faecium* to the extracellular matrix of diverse human cell groups (24). In addition, the presence of transmembrane proteins and an ABC transporter ATPase, might provide a putative mechanism for the translocation of this TM0504-like peptide.

Escherichia coli O157:H7. The infamous *E. coli* O157:H7 is a well-known threat to public health and has been implicated in fatal outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome (30). In the U.S., more than 73,000 infections and 63 deaths due to *E. coli* O157:H7 are reported each year. It has been previously shown that tmRNA is over-represented as a site of integration of phages in enterobacteriaceae (38). *E. coli* strains K-12 and CFT073 are not exceptions to this and have prophage insertions at the 3'end of the tmRNA. It is also known that these insertions affect the 3' of the tmRNA (15), which may inactivate the potential signaling peptide function on these strains. It is also known that, in *E. coli*, deletion or insertion mutants of tmRNA are associated with a reduction in growth rate and marked deficiencies in the response to heat shock (16, 28). Therefore, under laboratory conditions, the role of both the tmRNA and the putative TM0504 like peptide may be dispensable.

In all *E. coli* strains, putative small ORFs can be identified using the localization of TM0504 on *T. maritima* tmRNA as a guide. The most promising candidate of a biological relevant peptide is a small ORF with a relatively low isoelectric point (pI 5.24) (VELAGVEPASEIPTSSVLHA). Dosing experiments using a synthetic peptide based on this putative peptide (i.e., EIPTSSVLHA) in *E. coli* K-12. However, neither reduction in growth rate, morphological changes, nor Calcofluor-stainable EPS was observed. No further attempts to study potential effects at the transcriptional level were pursued. Nevertheless, the presence of a nearby hypothetical protein ECs348 (See Figure A.2) is noteworthy. This protein has some similarity to polyketide cyclases, which are involved in the circularization of linear poly-β-ketones. Interestingly, this gene is located nearby tmRNA and encoded in the same direction as the TM0504-like peptide, in several

representatives of the gamma-proteobacteria, including: *Enterobacteriacea*: *Erwinia; Yersinia*; *Shigella; Wigglesworthia; Salmonella; Photorhabdus; Vibrionales: Vibrio; Photobacterium; Coxiellacea; Pseudomonadaceae;* and *Synechococcus elongatus*. The possibility of post-translational "circularization" modifications and the use of an *E. coli* without tmRNA/phage insertions merit further examination.

Lactobacillus acidophilus NCFM. *L. acidophilus* is a gram-positive, homofermentative, catalase-negative rod. Initially isolated from humans, it is widely used as a probiotic strain in the production of yogurt, dietary supplements, juices, dried and fluid milk (36). This fastidious auxotrophic microorganism possesses desirable properties that might be used to promote human health. *L. acidophilus* NCFM Is amenable to genetic manipulations, produces bacteriocins, is able to survive in the harsh conditions in the human gastrointestinal tract, and is associated with health benefits, including a reduction of risk to colon cancer and incidence of pediatric diarrhea (2, 36).

In the available sequence of *L. acidophilus* NCFM, small peptides can be identified in both strands within the tmRNA. These putative peptides have a relative high pI (above of pH 9.8.). The peptide with the most similar location to the TM0504 peptide in *T. maritima* corresponds to the 26aa sequence: MEMRGIEPLS-KRISSLTSTIIVILLKLH. This peptide has a pI of 9.9; after a region conserved among all putative 3' TM0504-like (underlined) is removed, the pI change to 11.13. Similar changes in the isoelectric point have been seen for signaling peptides and some bacteriocins (7, 26).

Based on it genomic sequence, the tmRNA is flanked by an acetate kinase and an unknown hypothetical protein (LBA0742) (see Figure A.3) (2). Acetate kinase is a key enzyme responsible for de-phosphorylation of acetyl phosphate with the concomitant production of acetate and ATP. The carbon flux directed to acetate seems physiologically advantageous to some microorganisms, facilitating a faster growth rate and higher cell densities (8). It is intriguing also the co-localization of a competence-related type II secretion system cluster, including ComGF, ComGC, CglA, similar to a recently described *comY* operon (23). This operon is responsible for competence in *Streptococcus mutants* (23). It is important to emphasize that this *comY* operon also includes an acetate kinase. However, a relationship between the activity of an acetate kinase and competence has not been described. Nevertheless, this conformation seems to be phylogenetically conserved in *Lactobacillus johnsonii*. Moreover, natural competence have been reported in lactic acid bacteria, including the related *Lactobacillus lactis* (22). Hence, the relationship between the co-localization of the tmRNA, a competence cassette, raises interesting hypothesis for future studies.

On the other hand, downstream to the aforementioned region is a two-component system. This system shares significant sequence similarity at the protein level with the SrrA (46% identity and 65% similarity), a well-characterized two component system, which is involved in the global regulation of staphylococcal virulence factors in response to environmental oxygen levels (31). It might be interesting to explore the role of this system, relative to the *L. acidophilus* AP-tmRNA, under similar anaerobic conditions.

Lactococcus lactis subsp. lactis I11403. *L. lactis* ILL403 is a gram-positive facultative anaerobic bacterium, with a very limited biosynthetic capacity, but able of high specific growth rates when grown at optimal conditions (12). *L. lactis* ILL403 is widely used in the dairy industry, especially in soft cheese production. Based on the genomic sequence available (see Figure A.4), its tmRNA is flanked by the pi3 pro-phage, and a putative transcriptional regulator (YofM) (4). YofM has some similarity to YlxM, which is a transcriptional regulator implicated in the signal recognition translocation pathway (SRP) in *Streptococcus mutans* (17). Interestingly, contiguous to this ORF, and in the same orientation as the putative TM0504-like peptide, is a two-component system. Evidence suggests that this is a two-component system, also present in a related strain (*Lactococcus lactis* MG1363). The system is up-regulated during mid-exponential phase, with its highest level of expression during stationary phase (27). It could be interesting to explore the relationship between this cell dependant expression of the two component system and the proposed *T. maritima* like signaling molecule identified in this bacterium.

Mycoplasma pneumoniae M129. *M. pneumoniae* is among the smallest self-replicating organisms. This bacterium colonizes human respiratory tract epithelial cells causing tracheobronchitis and “atypical” pneumonia, a more acute form of pneumonia that progress more quickly with severe early symptoms. The infection process of *Mycoplasma* proceeds through attachment of bacteria to the host cell via specialized surface proteins, adhesins, and subsequent invasion can result in prolonged intracellular persistence that may cause cell death (35).

Examination of the *Mycoplasma* genomic data indicates the effect of evolutionary pressure to reduce the genome size of this parasitic bacterium. The genome of *M. pneumonia* lacks all of the genes involved in amino acid and cofactor biosynthesis, cell wall biosynthesis, lipid metabolism and also genes involved in important cellular processes, such as cell division, heat shock response, regulatory genes, two-component signal transduction systems, and most transcription factors (11). Interestingly, tmRNA is is present in the genome of all Mycoplasma strains sequenced thus far. Moreover, transposon mutagenesis experiments suggests that tmRNA is an essential gene in these microorganisms (13). The analysis of the tmRNA region of *M. pneumoniae* suggests the presence of a biologically significant AP-tmRNA peptide (see Figure A.4). tmRNA is flanked by MPN468 and MPN469, a cytoadhesin and a transmembrane protein, respectively (see Figure A.7).

Encoded within the tmRNA are several putative ORFs, most of which are small in size and with a relative low pI. One peptide of 42 aa that have a higher pI 10.3 (MRIKLNQLTPSHKLWHEPNYGDSIRVALVCSKRKRSWYLLVG). Note that this sequence possesses two proline residues (Pro10 and Pro18) that could be in principle recognized by MPN470, a nearby X-Pro peptidase, perhaps involved in the processing of the leader sequence and synthesis of the TM0504-like peptide.

***Streptococcus mutans* UA159.** Dental caries is one of the most common infectious diseases in humans. Between 200-300 bacterial species have been found to be associated with dental plaque. Only *Streptococcus mutans* has been consistently

implicated with the formation of dental caries (21). *Streptococcus mutans* strain UA159 was initially isolated from a child with active dental caries and its full genome sequence is available since 2002 (1).

Analysis of the tmRNA gene cluster in *S. mutans* reveals an apparent relationship between the TM0504-like peptide and some components of a putative ABC transporter constituted by SMU.1194 and SMU.1195. Contained within the tmRNA gene are two putative AP-tmRNAs with 26 and 36 aa, and isoelectric points of 5.96 and 9.06 respectively. No GG motif nor interesting feature can be reported, however three cysteine residues in the carboxi-terminal end in the longer putative peptide can be found (see Figure A.5).

***Xanthomonas oryzae pathovar oryzae* KACC1033** *X. oryzae pv. oryzae* KACC10331 is a gram-negative bacterium responsible for leaf blight disease in rice plants. In some conditions, fields can be infected with this strain, resulting in losses up to 50% in production. The genome sequence of this bacterial strain has been recently published (19). Based on the available data, the tmRNA is flanked by lipoprotein X003161 and a putative protease XOO3162 (see Figure A.5). According to the MEROPS database (34), X003161 belongs to the protease families S8 and S53 and, therefore, it is likely a specific peptidase that catalyze the release the last three amino acids of the amino-terminal end of the substrate protein (34). The putative TM0504-like peptide at the 3' end is a short peptide of 20 residues of sequence VEVGGIEPPSGSTPSPALHA. Interestingly, located downstream of the tmRNA and in the same orientation of this putative peptide a fully functional Gum gene cluster can be identified. This cluster is

responsible for EPS xantham production, which is an essential virulence factor in this bacterium (5). It would be interesting to examine the association of tmRNA and the putative peptide on the complementary strand and the synthesis of EPS in this bacterium. Perhaps, some similarities could be found between this set of genes and to the previously identified aggregation and EPS production mechanism as described in *T. maritima* (14).

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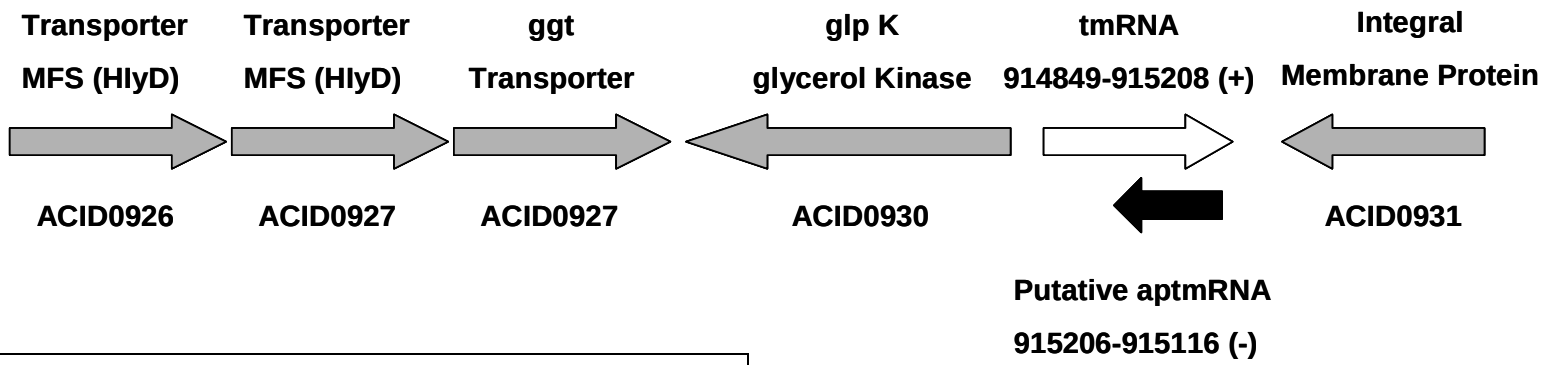
Acinetobacter sp. ADP1



Bacillus thuringiensis sp. konkukian

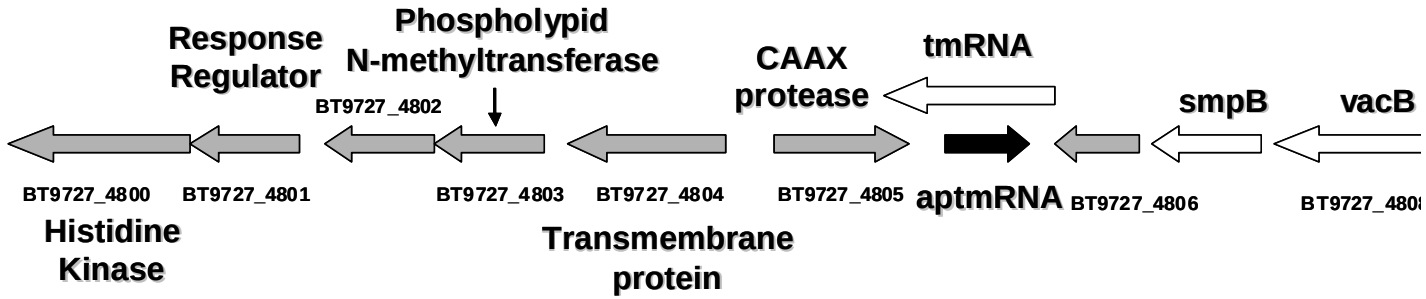
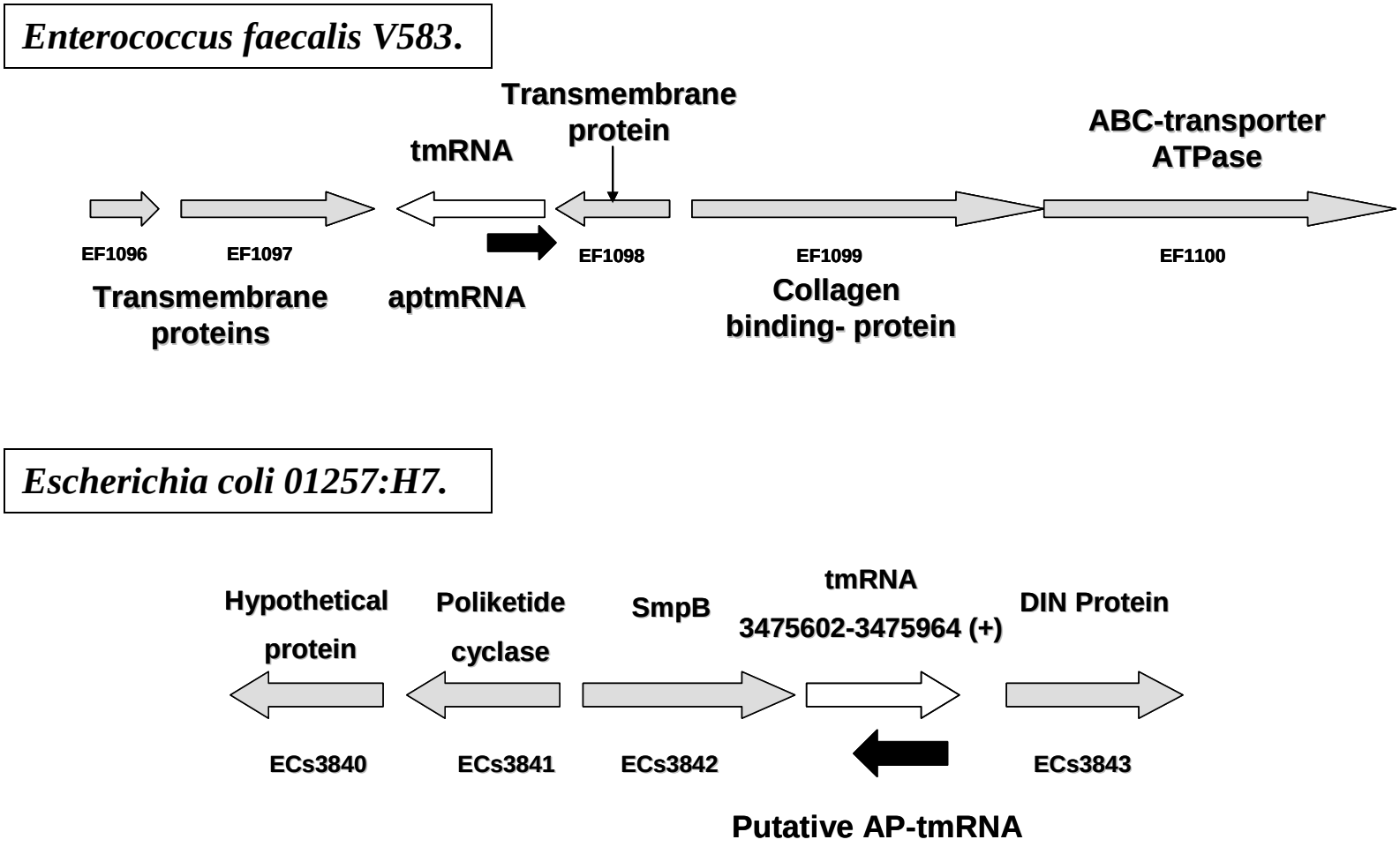


Figure A.1: Genomic organization of *Acinetobacter sp.* ADP1 and *Bacillus thuringiensis sp. konkukian* tmRNA region.



FigureA.2: Genome organization of the tmRNA locus in *Enterococcus faecalis V583* and *Escherichia coli 01257:H7.*

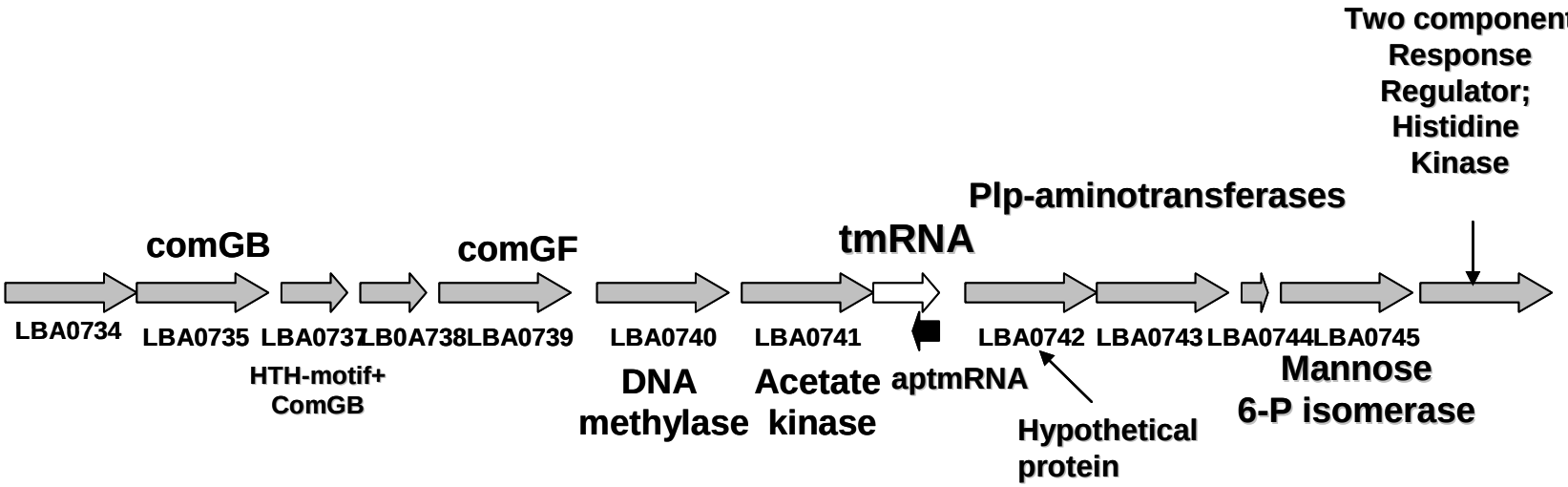
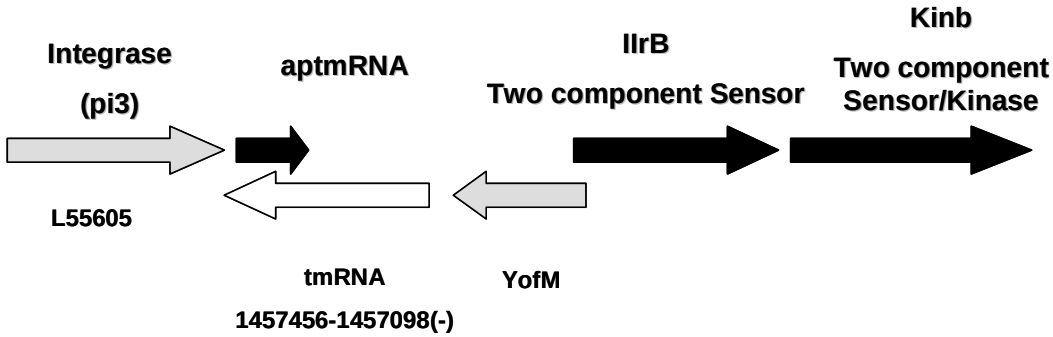


Figure A.3: Genomic organization of the tmRNA locus in *Lactobacillus acidophilus NCFM*.

Lactococcus lactis I11403



Mycoplasma pneumoniae M129

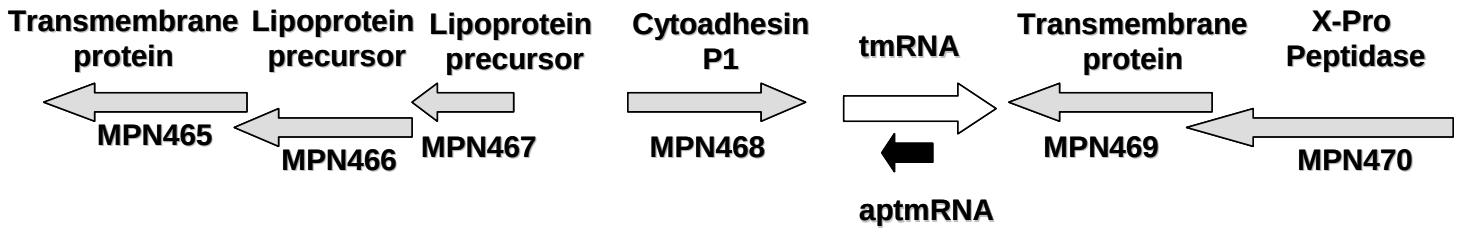


Figure A.4: Genomic organization of the tmRNA locus in *Lactococcus lactis I11403*. and *Mycoplasma pneumoniae M129*

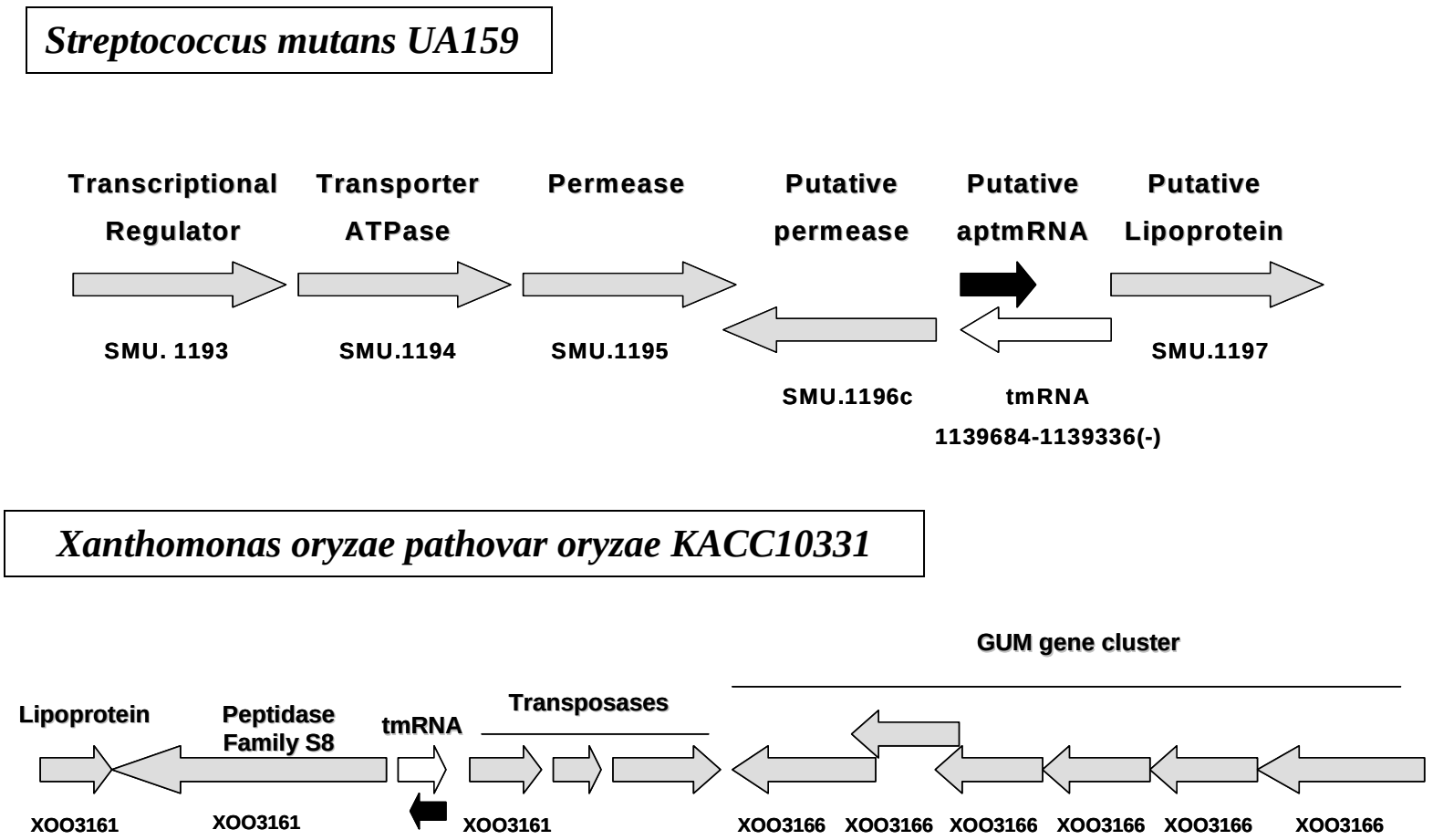


Figure A.5: Genomic organization of the tmRNA locus in *Streptococcus mutans UA159* and *Xanthomonas oryzae pathovar oryzae KACC1*

APPENDIX B:

Annotation of the AstB/ChuR genes in *T. maritima*

As annotated in the genome of *T. maritima* TM1317 belongs to the AstB/ChuR family of regulator proteins. However, upon closer examination we found that AstB is usually associated with succinyl-arginine hydrolases that are involved in catabolic pathways of utilization of arginine, an energy rich amino acid that can supply nitrogen, carbon and energy for different bacterial groups (7). However, AstB have no sequence homology or similar domains whatsoever when compared to TM1317. On the other hand, genes such as: *aslB* an arylsulfatase activating enzyme from *E. coli* (6), *atsB* from *Klebsiella* (5) and *chuR* from *Bacterioides thetaiomicron* (1, 2) belong to the same SAM_Radical superfamily. These genes share more sequence similarity (and more importantly, conserved domains) to TM1317, TM1300, TM1324, TM1325 which were differentially transcribed in *Thermotoga maritima* grown on *Pyrococcus* spent LPM

The gene *chuR* from *Bacteroides thetaiotaomicron* (homolog to BF3170 in *B. fragilis*), has been implicated in regulation of the utilization of sulfated mucopolysaccharides. A ChuR mutant of *B. thetaiotaomicron* was found to be unable to grow on sulfated mucopolysaccharides, such as chondroitin sulfate or on heparin. This finding was unexpected because these pathways were apparently independently regulated (1, 2). Interestingly, this observation was accompanied by a significant reduction of the enzymatic activity for the chondroitin-6-sulfatase. The original paper of identification of ChuR concluded that this protein had a regulatory role (1). However, a more plausible notion was presented by Fang (4). ChuR is proposed to be a sulfatase-modifying enzyme that modify at least the two sulfatases described in *Bacterioides sp* associated with the degradation of chondroiting-6 sulfates or the sulfated polysaccharide of heparin. The

arylsulfatases characterized so far require a formylglycine residue in order to become catalytically active (4, 5). The formylglycine is formed post-translationally from a cysteins or serine residue present in a conserved domain (3). Members of the AstB/ChuR family are responsible of these modifications (4), it is suggested that in *T. maritima* the annotation of AstB/ChuR regulatory proteins be changed to the more appropriate AslB/ChuR or AtsB/ChuR family of proteins.

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