Transcriptional Modulation of Penicillin-Binding Protein 1b, Outer Membrane Protein P2 and Efflux Pump (AcrAB-TolC) during Heat Stress Is Correlated to Enhanced Bactericidal Action of Imipenem on Non-typeable Haemophilus influenzae

CHERKAOUI, Abdessalam, et al.

Abstract

Objective: The purpose of the present study was to investigate the penicillin binding proteins (PBPs), drug influx and efflux modulations during heat stress and their effects on the bactericidal action of imipenem on non-typeable Haemophilus influenzae (NTHi). Methods: The two NTHi clinical isolates (GE47 and GE88, imipenem MICs by E-test > 32 μg/mL) examined in this study were collected at Geneva University Hospitals. The imipenem killing activity was assessed after incubation of the NTHi strains at either 37 or 42°C for 3 h with increasing concentrations of imipenem. The detection of PBPs was carried out by Bocillin-FL. Global transcriptional changes were monitored by RNA-seq after pre-incubation of bacterial cells at either 37 or 42°C, and the expression levels of relevant target genes were confirmed by qRT-PCR. Results: Quantitation of NTHi viable cells after incubation with 0.25 μg/mL of imipenem for 3 h revealed more than a twofold decrease in GE47 and GE88 viable cells at 42°C as compared to 37°C. Transcriptome analysis showed that under heat stress conditions, there were 141 differentially expressed genes […]

Reference

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Transcriptional modulation of penicillin-binding protein 1b, outer membrane protein P2 and efflux pump (AcrAB-TolC) during heat stress is correlated to enhanced bactericidal action of imipenem on nontypeable *Haemophilus influenzae*

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**Supplementary Materials**
Supplementary Fig.S1: Imipenem MICs determination using E-test method

The imipenem MIC for both strains was greater than 32µg/mL
Amino acid substitutions identified in the transpeptidase domain of the \textit{ftsI} gene (encoding for PBP3)

*shows the position of the catalytic serine residue

**Supplementary Table S1:** Amino acid substitutions in the \textit{ftsI}, \textit{dacB}, and \textit{acrR} genes for two imipenem resistant nontypeable \textit{H. influenzae} strains (GE47 and GE88)
### Supplementary Table S2: Susceptibilities of GE47 and GE88 to 14 antibiotics

* EUCAST clinical breakpoints for intravenous drug administration
** EUCAST clinical breakpoints for infections other than meningitis

| Antibiotic                  | Strain ID | MIC µg/ml | EUCAST clinical breakpoints | CLSI clinical breakpoints |
|-----------------------------|-----------|-----------|----------------------------|---------------------------|
| Imipenem                    | GE 47     | >32       | R                          | R                         |
|                             | GE88      | >32       | R                          | R                         |
| Ampicillin                  | GE 47     | 0.19      | S                          | S                         |
|                             | GE88      | 0.19      | S                          | S                         |
| Amoxicillin / clavulanic acid | GE 47     | 1.5       | S                          | S                         |
|                             | GE88      | 0.125     | S                          | S                         |
| Piperacillin/ mezlocillin    | GE 47     | 0.023     | S                          | S                         |
|                             | GE88      | 0.016     | S                          | S                         |
| * Ceferoxime                | GE 47     | 3         | R                          | S                         |
|                             | GE88      | 0.75      | S                          | S                         |
| Cefotaxime                  | GE 47     | 0.047     | S                          | S                         |
|                             | GE88      | 0.047     | S                          | S                         |
| Ceftixime                   | GE 47     | 0.032     | S                          | S                         |
|                             | GE88      | 0.032     | S                          | S                         |
| Ceftriaxole                 | GE 47     | 0.064     | S                          | S                         |
|                             | GE88      | 0.064     | S                          | S                         |
| Ertapenem                   | GE 47     | 0.594     | S                          | S                         |
|                             | GE88      | 0.25      | S                          | S                         |
| ** Meropenem                | GE 47     | 0.19      | S                          | S                         |
|                             | GE88      | 0.75      | R                          | R                         |
| Levofloxacin                | GE 47     | 0.023     | S                          | S                         |
|                             | GE88      | 0.023     | S                          | S                         |
| Co-amoxicilazol             | GE 47     | 4         | R                          | R                         |
|                             | GE88      | >32       | R                          | R                         |
| Clarithromycine             | GE 47     | 12        | I                          | I                         |
|                             | GE88      | 24        | I                          | I                         |
| Benzylpenicillin (screen 1 unit) | GE 47     | 11 mm     | R                          |                           |
|                             | GE88      | 6 mm      | R                          |                           |
| Ampicillin (2 µg)           | GE 47     | 14 mm     | R                          |                           |
|                             | GE88      | 6 mm      | R                          |                           |
| Imipenem (10 µg)            | GE 47     | 6 mm      | R                          | R                         |
|                             | GE88      | 10 mm     | R                          | R                         |
Supplementary Fig. S2: (A) Bacterial cell viability and (B) growth curves of GE47 and GE88 strains at either 37°C or 42°C in XV-supplemented brain heart infusion broth (sBHI). The inoculum suspension was prepared by picking several colonies from an overnight growth on chocolate agar plates and suspending the colonies in sBHI to a McFarland 0.5 standards density. Inoculated sBHI media were incubated at either 37 or 42°C. Values represent the mean ± SD from 2 independent biological replicates.
The longer incubation time at 42°C that did not significantly affect cell growth was 3h; this incubation time was then used in all experiments.

**Supplementary Fig.S3**: Assay procedure for viability measurement of NTHi cells after incubation with increasing concentrations of imipenem at either 37 or 42°C.
| Target | Gene | Sequence | References |
|--------|------|----------|------------|
| PBP1a  | ponA | Forward  | TCGGCAGGACAAATTTGGATT |
|        |      | Reverse  | AAGCCACCAGACCACTGCTTC | This study |
|        |      | Probe    | GCGTGCTAATGGGGAATGGCA |
| PBP1b  | ponB | Forward  | CGGTACAGGGCGGAAGTACG |
|        |      | Reverse  | CAAGGCTTCGTGCTTTGC | This study |
|        |      | Probe    | TTTATCGCGCAGACACCA |
| PBP2   | pbp2 | Forward  | GGTATGCAACGGGGGATTTGA |
|        |      | Reverse  | CCGAAATCGATCGCTTGC | This study |
|        |      | Probe    | CGGCTGCCAAATATACCAACCTGAGA |
| PBP3   | ftsI | Forward  | CCGCCAGTTATTGGGAACG |
|        |      | Reverse  | TTTACGCCGACACGTTAGCC | This study |
|        |      | Probe    | GCAATTTAAAAATAACGCGCAATGGT |
| AcrB   | acrB | Forward  | AGTTTCTTATCTGGTGCGACAGTTAC |
|        |      | Reverse  | ATCTCGTTTTACCTGCAATGAC | This study |
|        |      | Probe    | TGTGGATGTGGATGACCGCTTA |
| AcrR   | acrR | Forward  | GCGACAGATCGTTAAATGGCAAG |
|        |      | Reverse  | GGTAAATCGTTCCTGCAGGCTA | This study |
|        |      | Probe    | TGCTCAAACCTGCAAGAAGC |
| Omp2   | omp2 | Forward  | CGTGGGTGCATCGCACCTT |
|        |      | Reverse  | TCTGCAGAATGCTAAACGACCA | This study |
|        |      | Probe    | CACGAGCAAACGCAGCTT |
| RsmH*  | rsmH | Forward  | GGGCAAAATGGACGGTTATTTT |
|        |      | Reverse  | AAAACCGTTCTGCTTCATCA | This study |
|        |      | Probe    | TTGATCCTTGGTGTTGCTTCCTCAGC |

**Supplementary Table S3:** List of the primers designed and used in this study

*Ribosomal RNA small subunit methyltransferase H (originally designated as MraW).*
Supplementary Fig. S4: Percentage of viable NTHi cells relative to the control condition after exposition to increasing concentration of imipenem during 3h at either 37 or 42°C.

The amounts of GE47 and GE88 viable cells after incubation with increasing concentration of imipenem ranged from 0.25 to 256 µg/mL at either 37 or 42°C were normalized based on their amount of viable cells in a control condition (i.e. growth at either 37 or 42°C with 0µg/mL of imipenem).

(A): GE47 strain (imipenem MIC by E-test = >32 µg/mL)
(B): GE88 strain (imipenem MIC by E-test = >32 µg/mL)

Experiments were performed in 8 independent biological replicates.
* = p <0.05, ** =p <0.01 (paired Student's t test).
Supplementary Fig.S5

A: MDS plot with top 500 genes with the highest standard deviation in expression between samples.

B: MDS plot with top 500 genes with the highest fold change in expression between samples. The distance between each pair of samples is the root-mean-square deviation (Euclidean distance) for the top genes. Distances on the plot can be interpreted as leading log2-fold-change, meaning the typical (root-mean-square) log2-fold-change between the samples for the genes that distinguish those samples.
Supplementary Fig. S6: Volcano plot of differentially expressed genes

(A) chaperon proteins,  (B) division cell wall (dcw) gene cluster

Gray = genes that do not change in significantly expression; black = genes with expression values associated with FDR-corrected p-values < 0.05; blue = genes expression values associated with with FDR-corrected p-value < 0.05 and linear fold change between 37°C and 42°C higher than 2.
| Reference genome: *H. influenzae* Rd KW20 (NC_000907.1) | Gene | Function |
|----------------------------------------------------------|------|----------|
| **GE47 strain - Growth at 37°C** | SET-1 | SET-2 |
| Total reads | 12471697 | 12706187 |
| Mapped reads (97.9%) | 9958912 | 15852996 |
| **GE47 strain - Growth at 42°C** | SET-1 | SET-2 |
| Total reads | 13245098 | 13125243 |
| Mapped reads (81.9%) | 16172459 | 15994253 |
| **Statistical analysis** | log2 (Fold change) | logCPM | P Value | FDR |
|----------------------------------------------------------|-----------------|--------|---------|-----|
| HI0002 - | Long-chain-fatty-acid--CoA ligase FadD15 | 5494 | 8181 | 20023 | 18520 | 1.13 | 9.98 | 1.9E-20 | 6.4E-19 |
| HI0039 mreD | Rod shape-determining protein MreD | 810 | 829 | 2514 | 2396 | 1.19 | 6.99 | 7.4E-12 | 9.9E-11 |
| HI0040 - | hypothetical protein | 4161 | 4470 | 11868 | 11032 | 1.01 | 9.26 | 1.4E-11 | 1.8E-10 |
| HI0041 xthA | Exodeoxyribonuclease III | 5722 | 6692 | 17412 | 16048 | 1.04 | 9.80 | 3.3E-20 | 1.1E-18 |
| HI0042 rluA_1 | Ribosomal large subunit pseudouridine synthase A | 2872 | 3887 | 11769 | 11112 | 1.38 | 9.15 | 6.9E-21 | 2.5E-19 |
| HI0043 - | hypothetical protein | 4324 | 5562 | 15100 | 15091 | 1.23 | 9.59 | 3.4E-18 | 9.5E-17 |
| HI0044.1 (HI0044.1) - | tRNA-Ser | 56 | 63 | 211 | 181 | 1.33 | 3.35 | 3.0E-10 | 3.3E-09 |
| HI0070 recN | DNA repair protein RecN | 5347 | 6631 | 20465 | 18782 | 1.33 | 9.94 | 4.8E-33 | 1.0E-30 |
| HI0075 nrdD | Anaerobic ribonucleoside-triphosphate reductase | 820 | 1048 | 2561 | 2467 | 1.05 | 7.07 | 1.0E-10 | 1.2E-09 |
| HI0086.1 - | tRNA-Cys | 1348 | 1664 | 6129 | 5126 | 1.52 | 8.09 | 4.2E-24 | 2.2E-22 |
| HI0086.2 - | tRNA-Gly | 92 | 148 | 483 | 457 | 1.60 | 4.52 | 4.4E-18 | 1.2E-16 |
| HI0086.3 - | tRNA-Leu | 1969 | 1781 | 8190 | 6745 | 1.59 | 8.48 | 8.5E-18 | 2.3E-16 |
| HI0086.4 - | tRNA-Lys | 294 | 258 | 965 | 729 | 1.21 | 5.46 | 6.0E-08 | 4.8E-07 |
| HI0088 thrB | Homoserine kinase | 5236 | 6948 | 3648 | 3775 | -1.09 | 8.70 | 2.3E-13 | 3.7E-12 |
| HI0089 thrA | Bifunctional aspartokinase/homoserine dehydrogenase 1 | 14718 | 23134 | 9572 | 11138 | -1.23 | 10.28 | 1.3E-19 | 3.9E-18 |
| HI0092 gntP | High-affinity glucone transporter | 1853 | 2704 | 1201 | 1360 | -1.20 | 7.24 | 4.0E-12 | 5.6E-11 |
| HI0113.3 (HI_r02) - | 23S ribosomal RNA | 10209 | 3970 | 2113 | 2498 | -2.09 | 8.76 | 4.7E-08 | 3.8E-07 |
| HI0103.5 (HI_r03) - | 16S ribosomal RNA | 2302 | 995 | 703 | 597 | -1.80 | 6.70 | 5.8E-07 | 3.8E-06 |
| HI0111.6 - | tRNA-Pro | 73 | 63 | 200 | 188 | 1.12 | 3.40 | 2.5E-07 | 1.8E-06 |
| HI0111.7 - | tRNA-His | 172 | 174 | 848 | 768 | 1.83 | 5.24 | 4.2E-24 | 2.2E-22 |
| HI0122 metC | Cystathionine beta-lyase MetC | 4098 | 7555 | 2509 | 3449 | -1.32 | 8.54 | 5.9E-10 | 6.2E-09 |
| HI0123.1 - | tRNA-Gly | 111 | 141 | 505 | 429 | 1.51 | 4.53 | 6.8E-17 | 1.6E-15 |
| Gene      | Function                                                                 |
|-----------|---------------------------------------------------------------------------|
| HI0123.2  | - tRNA-Leu                                                                |
| HI0136.1  | - tRNA-Asp                                                                |
| HI0139    | ompP2 Outer membrane protein P2 OmpP2                                     |
| HI0157    | fabH 3-oxoacyl-[acyl-carrier-protein] synthase 3                           |
| HI0189    | gdhA NADP-specific glutamate dehydrogenase                                |
| HI0220.4  | - 23S ribosomal RNA                                                       |
| HI0220.6  | - tRNA-Glu                                                                |
| HI0220.5  | - 16S ribosomal RNA                                                       |
| HI0223    | - rarD protein, putative                                                  |
| HI0255    | dapA 4-hydroxy-tetrahydricolinate synthase                                |
| HI0256    | - hypothetical protein                                                    |
| HI0274.1  | - tRNA-Val                                                               |
| HI0274.2  | - tRNA-Val                                                               |
| HI0274.3  | - tRNA-Val                                                               |
| HI0274.4  | - tRNA-Val                                                               |
| HI0287    | mtr Tryptophan-specific transport protein                                 |
| HI0319    | cmoA tRNA (cmoSU34)-methyltransferase                                     |
| HI0325    | - Na+/H+ antiporter family protein                                        |
| HI0331    | - hypothetical protein                                                   |
| HI0332    | recO DNA repair protein RecO                                              |
| HI0333    | rimD 23S rRNA (uracl(1939)-C(5))-methyltransferase RimD                   |
| HI0380.2  | - tRNA-Lys                                                               |
| HI0438    | comB competence protein B                                                |
| HI0443    | recR Recombination protein RecR                                          |
| HI0444    | topB DNA topoiseromerase 3                                               |
| HI0465    | serA D-3-phosphoglycerate dehydrogenase                                  |
| HI0548    | infA Translation initiation factor IF-1                                  |
| HI0561    | - OPT oligopeptide transporter protein                                   |
| HI0583    | cpdB 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase precursor |
| HI0584    | iaaH Indole-3-acetyl-aspartic acid hydrolase                             |
HI0595  arcC1  Carbamoyl kinase 1  5386  7570  1843  2244  -2.04  8.55  1.2E-33  3.4E-31
HI0596  arcB_3  Ornithine carbamoyltransferase, catabolic  5236  7670  1742  2307  -2.04  8.54  5.7E-29  6.9E-27
HI0601.2 (HI_r07)  -  16S ribosomal RNA  2389  958  712  678  -1.73  6.74  3.9E-06  2.2E-05
HI0601.6  -  tRNA-Ile  120  140  490  579  1.66  4.69  1.4E-19  4.4E-18
HI0601.7  -  tRNA-Ala  80  114  477  482  1.93  4.48  4.6E-26  3.8E-24
HI0601.3 (HI_r08)  -  23S ribosomal RNA  10484  4141  2187  2365  -2.15  8.79  1.1E-08  9.7E-08
HI0609.1  -  tRNA-Pro  71  52  174  185  1.14  3.28  9.9E-07  6.2E-06
HI0621.2 (HI_r10)  -  16S ribosomal RNA  2440  997  726  650  -1.78  6.77  1.5E-06  9.1E-06
HI0621.3 (HI_r11)  -  23S ribosomal RNA  10339  4054  2238  2423  -2.09  8.78  2.8E-08  2.4E-07
HI0630  rseB  Sigma E factor regulatory protein RseB precursor  3829  3375  12334  11009  1.29  9.21  6.9E-13  1.0E-11
HI0642.2  -  tRNA-Arg  1356  1632  4330  3799  1.06  7.76  2.8E-15  5.6E-14
HI0648  mdaB  Modulator of drug activity B  3707  5746  2151  1831  -1.61  8.18  3.8E-21  1.5E-19
HI0683  glpC  Anaerobic glycerol-3-phosphate dehydrogenase subunit C  1319  2078  5818  5262  1.34  8.12  6.2E-15  1.2E-13
HI0684  glpB  Anaerobic glycerol-3-phosphate dehydrogenase subunit B  1138  1748  4900  4902  1.40  7.93  1.1E-21  4.5E-20
HI0685  glpA  Anaerobic glycerol-3-phosphate dehydrogenase subunit A  1572  2274  5829  5830  1.23  8.22  2.5E-15  5.0E-14
HI0689  glpQ  Glycerophosphoryl diester phosphodiesterase precursor  37728  41704  20958  21348  -1.30  11.36  1.1E-32  2.1E-30
HI0693  hel  Lipoprotein E precursor  25668  31158  16726  17520  -1.11  10.92  7.7E-26  5.9E-24
HI0723.1 (HI_r13)  -  16S ribosomal RNA  2305  989  725  625  -1.75  6.71  1.3E-06  7.8E-06
HI0723.2  -  tRNA-Ile  138  140  473  537  1.47  4.65  1.4E-15  2.9E-14
HI0723.5  -  tRNA-Ala  85  101  451  466  1.92  4.42  1.2E-25  9.0E-24
HI0723.3 (HI_r14)  -  23S ribosomal RNA  10341  3997  2203  2439  -2.09  8.77  4.0E-08  3.3E-07
HI0749  lexA  LexA repressor  4203  4766  14432  12528  1.20  9.44  1.1E-15  2.3E-14
HI0761.1  -  tRNA-Phe  17  19  56  53  1.21  1.62  1.1E-04  4.7E-04
HI0761.2  -  tRNA-Asn  74  82  256  252  1.32  3.72  1.1E-11  1.4E-10
HI0775  cynR_1  HTH-type transcriptional regulator CynR  1474  1842  853  875  -1.32  6.76  2.9E-15  5.7E-14
HI0779  rplW / rpl23  5OS ribosomal protein L23  34471  37692  23149  21874  -1.07  11.29  2.1E-22  9.5E-21
HI0781  rpsS19  30S ribosomal protein S19  30786  36301  22110  21318  -1.01  11.20  2.8E-22  1.2E-20
HI0818  galM  Aldose 1-epimerase  2070  3248  11696  11430  1.76  9.07  6.8E-27  6.7E-25
HI0819  galK  Galactokinase  2121  3135  10534  9905  1.59  8.93  3.9E-24  2.1E-22
HI0822  mglB  D-galactose-binding periplasmic protein precursor  31743  40153  23879  22643  -1.01  11.29  9.2E-23  4.5E-21
| Gene      | Description                                                                 | Gene      | Description                                                                 | Value 1 | Value 2 | Value 3 | Value 4 |
|-----------|------------------------------------------------------------------------------|-----------|------------------------------------------------------------------------------|---------|---------|---------|---------|
| trmA      | tRNA/tmRNA (uracil-C(5))-methyltransferase                                   |           |                                                                              |         |         |         |         |
| hbpA_1 / dppA | Heme-binding protein A precursor                                           |           |                                                                              |         |         |         |         |
| clpB      | Chaperone protein ClpB                                                      |           |                                                                              |         |         |         |         |
| pepB_2    | Peptidase B                                                                 |           |                                                                              |         |         |         |         |
| panF      | Sodium/pantothenate symporter                                               |           |                                                                              |         |         |         |         |
| rpmH / rpL34 | 505 ribosomal protein L3                                                   |           |                                                                              |         |         |         |         |
| npA       | Ribonuclease P protein component                                            |           |                                                                              |         |         |         |         |
| yidD      | Putative membrane protein insertion efficiency factor                       |           |                                                                              |         |         |         |         |
| yidC      | Membrane protein insertase YidC                                             |           |                                                                              |         |         |         |         |
| ahpD      | Alkyl hydroperoxide reductase AhpD                                          |           |                                                                              |         |         |         |         |
| oppF      | Oligopeptide transport ATP-binding protein OppF                             |           |                                                                              |         |         |         |         |
| oppD_1    | Oligopeptide transport ATP-binding protein OppD                             |           |                                                                              |         |         |         |         |
| oppC      | Oligopeptide transport system permease protein OppC                         |           |                                                                              |         |         |         |         |
| oppB      | Oligopeptide transport system permease protein OppB                         |           |                                                                              |         |         |         |         |
| oppA_2    | Periplasmic oligopeptide-binding protein precursor                          |           |                                                                              |         |         |         |         |
| talB      | Transaldolase B                                                            |           |                                                                              |         |         |         |         |
| serC      | Phosphoserine aminotransferase                                              |           |                                                                              |         |         |         |         |
| artM      | Arginine ABC transporter permease protein ArtM                             |           |                                                                              |         |         |         |         |
| artP      | Arginine transport ATP-binding protein ArtP                                |           |                                                                              |         |         |         |         |
| sucD      | Succinyl-CoA ligase [ADP-forming] subunit alpha                             |           |                                                                              |         |         |         |         |
| -         | putative TonB-dependent receptor precursor                                  |           |                                                                              |         |         |         |         |
| -         | translation initiation factor Sui1                                          |           |                                                                              |         |         |         |         |
| trigC     | Glucose-1-phosphate adenyltransferase                                       |           |                                                                              |         |         |         |         |
| glgA      | Glycogen synthase                                                           |           |                                                                              |         |         |         |         |
| -         | tRNA-Asn                                                                    |           |                                                                              |         |         |         |         |
| -         | NAD(P) transhydrogenase subunit alpha                                       |           |                                                                              |         |         |         |         |
| -         | NAD(P) transhydrogenase subunit beta                                        |           |                                                                              |         |         |         |         |
| -         | Phosphate regulon transcriptional regulatory protein PhoB                   |           |                                                                              |         |         |         |         |
| Gene  | Function                                      | Coordinates | MAF  | Blat  | Log2 Fold Change |
|-------|-----------------------------------------------|-------------|------|-------|------------------|
| HI1380| pstB Phosphate import ATP-binding protein     | 9812        | 11190| 5030  | 4905 -1.47       | 9.39 3.9E-24 2.1E-22 |
| HI1381| pstA Phosphate transport system permease      | 11511       | 16809| 6184  | 6949 -1.48       | 9.80 2.9E-31 4.9E-29 |
| HI1382| pstC Phosphate transport system permease      | 19003       | 26948| 9759  | 11231 -1.50      | 10.49 1.0E-34 4.4E-32 |
| HI1383| pstS Phosphate-binding protein                | 31430       | 46702| 14672| 17950 -1.63      | 11.22 6.7E-34 2.3E-31 |
| HI1389.1| trpC yadA                                     | 3065        | 3869 | 8377  | 10120 1.04       | 8.95 6.0E-11 7.2E-10 |
| HI1403| - Phage tail fiber repeat protein             | 203         | 360  | 167   | 163 -1.13       | 4.23 3.8E-08 3.1E-07 |
| HI1405| - hypothetical protein                        | 42          | 60   | 29    | 35 -1.05       | 1.84 2.3E-04 8.7E-04 |
| HI1424.1| - tRNA-Leu                                    | 27          | 30   | 77    | 75 1.03        | 2.12 1.7E-04 6.6E-04 |
| HI1530| gltS Sodium/glutamate symport carrier protein| 30992       | 37971| 16818| 16506 -1.43      | 11.11 6.2E-43 5.2E-40 |
| HI1545| sstT Serine/threonine transporter             | 5471        | 7082 | 3881  | 3937 -1.06      | 8.76 6.3E-13 9.7E-12 |
| HI1546| - DNA polymerase V subunit                   | 549         | 551  | 1574  | 1358 1.02       | 6.30 3.7E-08 3.1E-07 |
| HI1601| - hypothetical protein                        | 1128        | 2449 | 543   | 775 -1.78       | 6.71 1.9E-11 2.4E-10 |
| HI1603| - Phosphate transport regulator               | 1624        | 1685 | 4809  | 5044 1.18       | 8.00 1.5E-16 3.4E-15 |
| HI1617| aspC Aspartate aminotransferase              | 3125        | 4219 | 1756  | 1831 -1.41      | 7.88 2.8E-25 1.9E-23 |
| HI1632| lysC Lysine-sensitive aspartokinase 3         | 6246        | 6826 | 2407  | 2514 -1.80      | 8.63 7.9E-31 1.1E-28 |
| HI1702| metE 5-methyltetrahydroperoylglutamate--      | 16947       | 20126| 11863| 12007 -1.02      | 10.34 1.4E-21 5.3E-20 |
| HI1707| qseC Sensor protein QseC                      | 4609        | 6296 | 2542  | 2426 -1.51      | 8.42 1.1E-22 5.1E-21 |
| HI1708| qseB Transcriptional regulatory protein       | 3916        | 5421 | 2012  | 1793 -1.67      | 8.15 3.8E-26 3.3E-24 |
| HI1709| - hypothetical protein                        | 8401        | 11751| 5183  | 4275 -1.46      | 9.32 9.2E-21 3.2E-19 |
| HI1725| pbp1B (ponB) Penicillin-binding protein 1B     | 2805        | 3694 | 9918  | 10162 1.25      | 9.00 3.0E-17 7.5E-16 |
| HI1728| mntH Divalent metal cation transporter        | 2454        | 4339 | 1371  | 1816 -1.45      | 7.73 2.3E-14 4.1E-13 |
| HI1729| - LamB/Ycsf family protein                   | 672         | 1410 | 219   | 371 -2.16      | 5.85 2.2E-13 3.5E-12 |
| HI1730| kipA Kipl antagonist                          | 903         | 2168 | 242   | 485 -2.41      | 6.36 2.0E-12 9.2E-11 |
| HI1731| kipl Kinase A inhibitor                       | 770         | 1466 | 310   | 396 -2.01      | 5.99 1.8E-17 4.7E-16 |
| HI1733| rnb Exoribonuclease 2                        | 3123        | 3841 | 9658  | 9924 1.11      | 9.01 3.1E-14 5.2E-13 |
| HI1739.6(HI_r17)| - 23S ribosomal RNA | 10053       | 3975 | 2195  | 2377 -2.08      | 8.74 2.6E-08 2.3E-07 |
| HI1739.5| - tRNA-Ala                                    | 84          | 96   | 442   | 465 1.95       | 4.40 1.3E-25 9.2E-24 |
| HI1739.4| - tRNA-Ile                                    | 134         | 138  | 517   | 598 1.64       | 4.75 3.2E-19 9.2E-18 |
| HI1739.3(HI_r18)| - 16S ribosomal RNA | 2327       | 996  | 723   | 641 -1.75      | 6.73 1.3E-06 7.7E-06 |
Supplementary Table S4: Summary statistics of RNA-seq profiles between 37°C and 42°C of the 141 differentially expressed genes with a $|\log_2(\text{fold change})| > 1$, including 67 up-regulated and 74 down-regulated.

LogCPM = Logarithm of counts per million reads
FDR = False discovery rate
