Transcription profiling data set of different states of 
*Mycoplasma gallisepticum*

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**ABSTRACT**

*Mycoplasma gallisepticum* belongs to class Mollicutes and causes chronic respiratory disease in birds. It has a reduced genome, lack of cell wall and many metabolic pathways, and also easy to culture and non-pathogenic to humans. Aforementioned made it is a convenient model for studying of systems biology of minimal cell. Studying the transcriptomic level of *M. gallisepticum* is interesting for both understanding of common principles of transcription regulation of minimal cell and response to definite influence for pathogen bacteria.

For rapid investigation of gene expression we developed microarray design including 3366 probes for 678 genes. They included 665 protein coding sequences and 13 antisense RNAs from 816 genes and 17 ncRNAs present in *Mycoplasma gallisepticum*. The study was performed on Agilent one-color microarray with custom design and random-T7 polymerase primer for cDNA synthesis.

Here we present the data for transcription profiling of *M. gallisepticum* under different types of exposures: genetic knock-out mutants, cell culture exposed to sublethal concentrations of antibiotics and well-characterized heat stress effect. Mutants have transposon insertion to hypothetical membrane protein, lactate dehydrogenase, helicase with unknown function, 1-deoxy-D-xylulose 5-phosphate reductoisomerase or potential sigma factor. For inhibition of important cell systems, treatment with carbonyl cyanide m-chlorophenylhydrazone (CCCP), novobiocin or tetracycline were chosen. Data are available via NCBI Gene Expression Omnibus (GEO) with the accession number GSE85777 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE85777).

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1. **Direct link to deposited data**

Data are available via NCBI Gene Expression Omnibus (GEO) with accession number GSE85777 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE85777).

2. **Experimental design, materials and methods**

2.1. **Experimental design**

For studying *Mycoplasma gallisepticum* as model object of minimal cell, there is task of rapid high-throughput transcription profiling of different state of this bacteria. We developed microarray design including 3366 probes for 678 genes (5 probes for each gene, when possible). They included 665 protein coding sequences and 13 antisense RNAs from 816 genes and 17 ncRNAs present in *Mycoplasma gallisepticum* S6. The used approach has allowed us to average possible variation in the representation of RNA fragments inside genes.

Microarray with selected oligos was made by Agilent technologies. Cy-3 modified cRNA was obtained using standard Agilent protocol and
primer composed from T7 RNA polymerase promoter and random hexamer. Despite the possible dominance of ribosomal RNA in samples, the good quality of gene expression data was produced.

This dataset provides gene expression data for genetic knock-out mutants of *Mycoplasma gallisepticum*, cell culture exposed to sublethal concentrations of antibiotics and under heat stress. The chosen mutants have insertion in 5′ UTR GCW_03380 (hypothetical membrane protein), RBS GCW_00390 (lactate dehydrogenase), GCW_03935 (helicase SNF2), GCW_00495 (1-deoxy-D-xylulose 5-phosphate reductoisomerase) and GCW_00440 (alternate sigma factor of RNA polymerase). Treatments were carry out with CCCP, tetracycline, novobiocin or thermal exposure under sublethal conditions for *M. gallisepticum*. Description of samples is presented in Table 1.

Quality of data is demonstrated on Fig. 1. Almost all obtained data do not exceed the coefficient of variability (CV) 10% for 80% of data, with average CV 8.5% (Fig. 1A). Boxplot with normalized data is on Fig. 1B. Data show good reproducibility as seen on Fig. 2. Spearman correlation coefficient between biological repeats ranges between 0.93 and 1.00. At heatmap the high similarity between all mutants and wild type *M. gallisepticum* is demonstrated.

Due to variability in the intensity of different probes inside a gene, further we calculate changes in intensity level for each probe and averaged it. It allowed us to estimate fold change of expression level more accurately.

Gene expression data obtained by hybridization on the microarray, was validated for 94 genes using qualitative PCR with reverse transcription for 3 samples with major changes. The obtained results correlate well with each other (Fig. 3), the observed shift of the trend line along the x-axis can be explained due to the difference in the normalization of samples measurements for both methods.

### 3. Materials and methods

#### 3.1. Cell culturing

*M. gallisepticum* S6 was cultivated on a liquid medium containing tryptose (20 g/l), Tris (3 g/l), NaCl (5 g/l), KCl (5 g/l), yeast dialysate (5%), horse serum (10%) and glucose (1%) at pH = 7.4 and 37 °C in aerobic conditions. Cells were passaged in 1:10 dilution twice for 24 h, starting from frozen culture prior to the experiment.

#### 3.2. Construction of mutants with random transposon insertion

Construction of a vector for transformation of *M. gallisepticum* was done as described in [1]. Transformation was performed by electroporation as described in [2].

#### 3.3. Determination of sub-lethal conditions

Sub-lethal conditions were determined as describe previously [3] as conditions when stressful actions are maximal but most of the cells are still viable. Cell viability was estimated by the determination of colony forming units by cells after stress.

#### 3.4. Stress exposures

The wild-type *M. gallisepticum* cells were treated with sub-lethal concentrations of CCCP (final concentration 50 μg/ml in culture media), novobiocin (50 μg/ml), tetracycline (8 μg/ml) during 1 h or were under 46°C during 15 min.

#### 3.5. RNA extraction

Total RNA was prepared using direct lysis of cell culture in exponential growth phase in TRizol LS reagent (Life Technologies) according to the manufacturer’s instructions. RNA was treated by DNase I (Thermo Scientific) and followed by ethanol precipitation. RNA was quantified using a Qubit 2.0 fluorometer.

#### 3.6. Microarray design

An oligonucleotide-based microarray specific for *M. gallisepticum* was designed. It represents 678 ORF including genes and ncRNA. For

| Sample name | Clone | Treatment |
|-------------|-------|-----------|
| C_1         | WT    | Cell culture in exponential growth phase |
| C_2         | WT    | Cell culture in exponential growth phase |
| Tn_SUTR_03380_1 | CR1, Transposon insertion to 5′ UTR of hypothetical protein GCW_03380 | Cell culture in exponential growth phase |
| Tn_SUTR_03380_2 | CR1, Transposon insertion to 5′ UTR of hypothetical protein GCW_03380 | Cell culture in exponential growth phase |
| Tn_RBS_00390_1 | CR19, Transposon insertion to RBS of lactate dehydrogenase GCW_00390 | Cell culture in exponential growth phase |
| Tn_RBS_00390_2 | CR19, Transposon insertion to RBS of lactate dehydrogenase GCW_00390 | Cell culture in exponential growth phase |
| Tn_03935_1 | CR30, Transposon insertion to helicase SNF2 GCW_03935 | Cell culture in exponential growth phase |
| Tn_03935_2 | CR30, Transposon insertion to helicase SNF2 GCW_03935 | Cell culture in exponential growth phase |
| Tn_00495_1 | CR86, Transposon insertion to 1-deoxy-D-xylulose 5-phosphate reductoisomerase GCW_00495 | Cell culture in exponential growth phase |
| Tn_00495_2 | CR86, Transposon insertion to 1-deoxy-D-xylulose 5-phosphate reductoisomerase GCW_00495 | Cell culture in exponential growth phase |
| Tn_00440_1 | 5.3, Transposon insertion to potential sigma factor GCW_00440 | Cell culture in exponential growth phase |
| Tn_00440_2 | 5.3, Transposon insertion to potential sigma factor GCW_00440 | Cell culture in exponential growth phase |
| cccp_1     | WT    | Cell culture in exponential growth phase under treatment with sublethal concentrations of CCCP |
| cccp_2     | WT    | Cell culture in exponential growth phase under treatment with sublethal concentrations of CCCP |
| novo_1     | WT    | Cell culture in exponential growth phase under treatment with sublethal concentrations of novobiocin |
| novo_2     | WT    | Cell culture in exponential growth phase under treatment with sublethal concentrations of novobiocin |
| tet_1      | WT    | Cell culture in exponential growth phase under treatment with sublethal concentrations of tetracycline |
| tet_2      | WT    | Cell culture in exponential growth phase under treatment with sublethal concentrations of tetracycline |
| HS_1       | WT    | Cell culture in exponential growth phase under heat stress at 46 °C during 15 min |
| HS_2       | WT    | Cell culture in exponential growth phase under heat stress at 46 °C during 15 min |
Fig. 1. Quality of microarray data. A – coefficient of variability (CV) for 80% of data between technical replicates of probes for each sample. Red line is on CV = 10%. B – boxplot for distribution of logarithm of normalized intensity for each sample.

Fig. 2. Similarity of gene expression between samples.
each ORF, when possible, 5 different probes (60-mer) were selected with following algorithm. For each gene a list of oligonucleotides of a fixed length was created and then filtered using desired range dG of duplex formation. Thermodynamics of hybridization was calculated using SantaLucia method. Then the oligonucleotides were tested for cross-hybridization. From corresponding oligonucleotides 5 probes with uniform distributions on the gene were selected. Total different probes on each slide are 3366. Each spots were printed 4 times on each slide to improve the reproducibility of array data. Microarray was made by Agilent Technologies (Custom Gene Expression Microarrays, 8 × 15 K).

3.7. Microarray experiment

Cyanine-3 (Cy3) labeled cRNA was prepared from 200 ng total RNA using the Low Input Quick Amp Labeling Kit, One-Color (Agilent) according to the manufacturer’s instructions except using primer 5′-AAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGCGCNNNNNNN-3′ instead T7 Primer, followed by PureLink RNA Mini Kit column purification (Thermofisher Scientific). Dye incorporation and cRNA yield were checked with the NanoDrop ND-1000 Spectrophotometer. 600 ng of Cy3-labeled cRNA (specific activity >12 pmol Cy3/μg cRNA) were
| SG_ID     | gene_name for rev | for | rev |
|-----------|------------------|-----|-----|
| GCW_02785 | gbp              | GAAAGATTACAGGCAAAAGGTG | GCCCTTCCTCCTCATTAGACTCC |
| GCW_03075 | dnaK             | TTATCCAGAAAGTCTTCTC   | TAACTTGAAGATCTCATTCAG |
| GCW_02105 | lon              | TTGATAGTGACCTTCTCTC   | ATCTCCCTAAGATCTTACAG |
| GCW_02710 | groEL            | GAAGATTACAGGCAAAAGGTG | GCCCTTCCTCCTCATTAGACTCC |
| GCW_00415 | dnaJ             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00845 | dnaJ2            | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_01610 | dnaJ4            | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_01620 | grpE             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_02005 | hrcA             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00085 | GCW_00085        | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_01140 | dps              | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_01335 | 16S              | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00390 | 23S              | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_02860 | gapd             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00160 | tktA1            | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00165 | osmC             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_03005 | GCW_03005        | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00065 | glpF             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00075 | glpO             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_02750 | acoA             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_02745 | acoB             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_02740 | aceF             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00395 | ldh              | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00390 | uvrB             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00440 | uvrA             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_03260 | parE             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_03255 | ruvA             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_03250 | ruvB             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00765 | ptsG1            | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_02325 | parC             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00205 | urvB             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |
| GCW_00210 | urvC             | TTCTTATGCTTCTCTCTC    | TGAAAGATCTCATTCAGACTCC |

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fragmented and hybridized to Agilent Custom Gene Expression Microarray, 8 × 15 K (G2509F) according to the manufacturer's instructions. Slides were scanned on the Agilent DNA Microarray Scanner (G2505B). The scanned images were analyzed with Feature Extraction Software 9.1 (Agilent) using default parameters and custom grid 069985_D_F_20140926. All further calculations were made using basic scripting in R. Fluorescence values of all of non-control probes on the microarray were normalizing using scaling normalization.

All experiments were carried out in two biological repeats. Data were deposited into the NCBI GEO repository under the accession number GSE85777.

3.8. Validation of microarray data by quantitative RT-PCR

cDNA was synthesized from random hexamer primers by H-minus Mu-MLV reverse transcriptase (Thermo Scientific). Real-time PCR was performed using iQ SYBR Green Supermix (Bio-Rad) and a CFX96 Real-Time PCR Detection System (Bio-Rad). Used primers are presented in Table 2. Quantitative data were normalized to the 23S rRNA transcript as described previously [3].

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