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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)

Specifications	
Organism/cell line/tissue	*Mycoplasma gallisepticum S6*
Sex	NA
Sequencer or array type	Agilent one-color custom gene expression microarray
Data format	normalized and processed microarray data, qPCR data
Experimental factors	transcriptional profiling of genetic knock-out mutants of *Mycoplasma gallisepticum*, cell culture exposed to sublethal concentrations of antibiotics and under heat stress.
Experimental features	the data were obtained on Agilent one-color microarray with custom design and random-T7 polymerase primer for cDNA synthesis.
Consent	NA
Sample source location	Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russian Federation

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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

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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 quantitative 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 describe 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 46C 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.

Table 1
Description of samples for transcription profiling.

Sample name	Clone	Treatment
C_1	WT	Cell culture in exponential growth phase
C_2	WT	Cell culture in exponential growth phase
Tn_5UTR_03380_1	CR1, Transposon insertion to 5′ UTR of hypothetical protein GCW_03380	Cell culture in exponential growth phase
Tn_5UTR_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

3.6. Microarray design

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

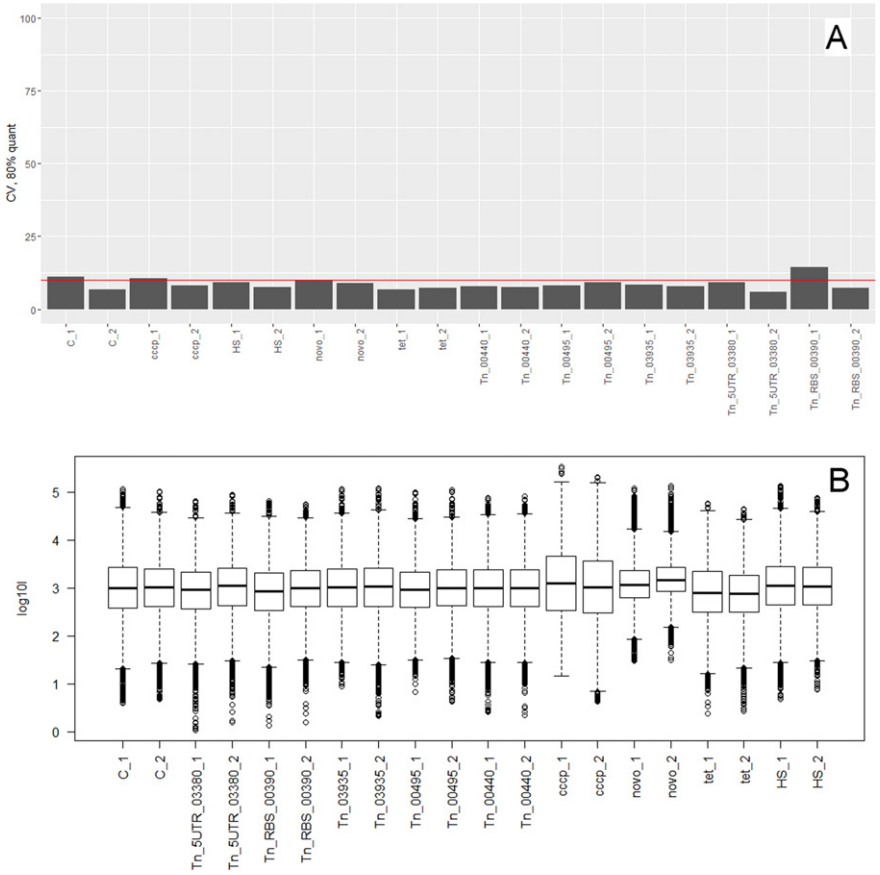

Fig. 1. Quality of microarray data. A – coefficient of variability (CV) for 80% of data between technical replicates of probes for each sample. Red line is on CV = 10%. B – boxplot for distribution of logarithm of normalized intensity for each sample.

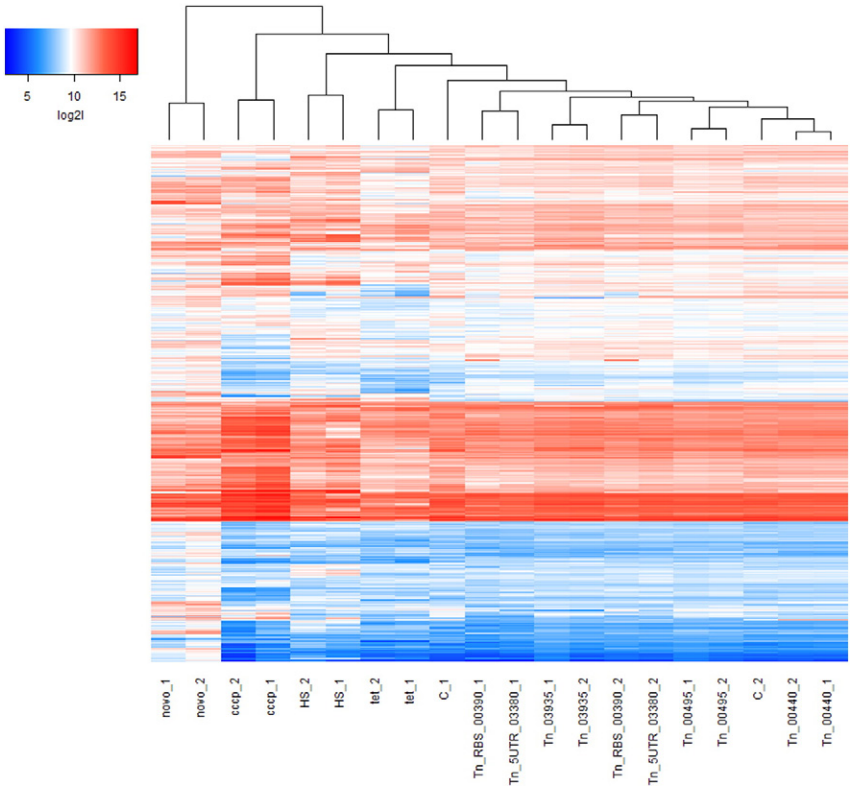

Fig. 2. Similarity of gene expression between samples.

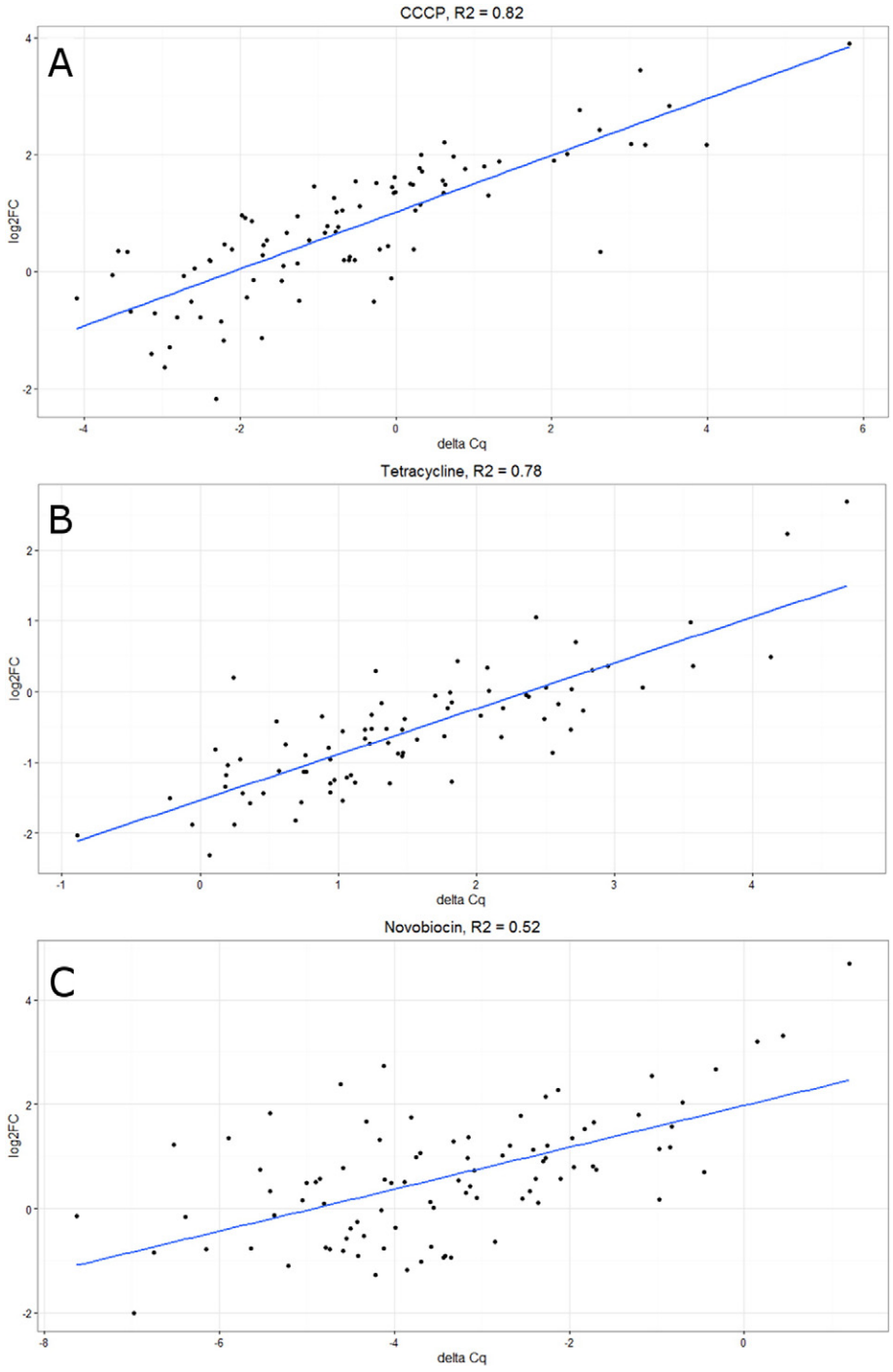

Fig. 3. Validation of microarray data by quantitative RT-PCR. Correlation of normalized fold expression from RT-qPCR (x-axis) and logarithm of fold changes genes from microarray analysis (y-axis). A – for CCCP treatment, B – for tetracycline treatment, C – for novobiocin treatment.

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′-AAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGCGCNNNNNN-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

Table 2
RT-qPCR primers.

S6_ID	gene_name	for	rev
GCW_02785	clpB	GAAAGATTACAGGCAAAAGGTG	GCGCTCTTCCTCTTAGTACTGC
GCW_03075	dnaK	TTACTCCAGAAGAAGTTTCTGC	TAACTTCGAAAGTACCATCAGC
GCW_02105	lon	TTGTTAAGATCTCACTTGGTGG	ATCCCTTCAACATAGTTAGCAG
GCW_02710	groEL	ACAACAGCTACAATCTTAACTC	CCCTTGAACTCTAAACCATCAG
GCW_00415	dnaJ	TCTTTAGCTTTGAAGGTGTGAG	GTGAAAGTAGTTCATCGTCTGC
GCW_00845	dnaJ2	CATGTAGTGATTGTAATGGCGA	GGTTATCGCGTAGTTCAAACAC
GCW_01610	dnaJ4	CCCGATGTTAATAAAGCTCCTG	TCAATCTTACGACTCATCTCAG
GCW_01620	dnaJ5	AGCTGAGTTTCAGATTGAAGAC	TAAGCTGATCTAACATCATCAC
GCW_01900	dnaJ6	GCGTATGAAGTTCTATCTAACCC	TTAGCTTGATTTGCACTAGCAC
GCW_04075	dnaJ7	AGTTAAGAGACTAATTAATCGACCAG	CAACATTACCCTTACCAAAGAACTC
GCW_01905	grpE	TTAGAGTTTGCTTCACTTGACC	GTTTGAGGTTGTTCTTGTTCAC
GCW_02005	hrcA	AATGATCGGTTAGTTGATACCC	TTCAATCCCAATATCTTGACCA
GCW_02100	tig	GAAAACCCAGAAGTTAGCGTAG	CTCTAATTCGTAGTTTTTCGCC
GCW_03080	GCW_03080	CAGATGCAATTACGGTTGTAGG	TGATTTACGTATTCCAAACGCT
GCW_02930	GCW_02930	GCAAAGTTACTGTAACACCAGG	CGGCATTAACCATTATTTCACC
GCW_00085	GCW_00085	CCAATATAATAGCGAAATGGCTG	GTTAATTAAAGCCACTTCAGCG
GCW_01140	dps	AACTTTCACTGAAATATCAAAGGTG	TTTCATCTAAGATTGGTTGAACACG
GCW_01335	16S	AAGTAACGACTAACTATGTGCC	TAGGGTATCTAATCCTATTTGCTCC
GCW_00395	23S	AGAAATACGTAGTCGATGGAAG	GAGTTCCTTAGCTATAGTTCACTC
GCW_02860	eno	GCGATCTTAGCAGTATCAATGG	ACCTTCATCACCTTTGTTAGTA
GCW_01780	gapd	ACGACTTAACTGATGCTAAGAC	TGGTTCACGTTGTAAACTACAG
GCW_03290	tpiA	TATTCGTACATTTGGCTTTGGC	CTGATCTTTAAGTACAGCTTTGGTC
GCW_01315	tuf	TCAAAATCGTCACTATGCACAC	TCTGATTACTGGAGTGTTATCACC
GCW_00475	tsf	AGTTAGCTGATCAAAAAGCAAC	TTAACAATAACTCCAATGCGGC
GCW_01635	osmC	AGCTAAAGTTGGTAGAGAAGGTC	ATTCTTTAGCCTTAGCTTCTGG
GCW_03005	GCW_03005	ATTCATCAGCAACTAAAGTACC	TCATCAATTACGATTCTTCCAG
GCW_00065	glpF	CAGCTAAAAGAATCATAGTGCC	GTGGTGTGGTTATTAGTATCCAAG
GCW_00075	glpO	GATGAATTGCTACTGAAGAGGTG	ATTCATGGTGGTTTTGATCCTG
GCW_90066	glpK	CTCGTTGAGATCACAAACTAGG	TCTCAGGGACTAAGATTCGTTG
GCW_01615	gpsA	TAATGTAGCTAAGGGAATGGACG	TGTTCAAAAACAATCCCAGTCC
GCW_02750	acoA	GTCTTCTGTGTAAACAACAACC	CGATTGGATCTGATTTCTTAGCTTC
GCW_02745	acoB	CTAAAGCTTTAATTCCACCACCC	GTATTATACGGTCAAGACGCTG
GCW_02740	aceF	TAAACCGATCTTCTTAGAGCGAG	TACCGTAAGCAAGTTAAGGATG
GCW_00390	ldh	CGATTACTGCTTCAATTCCAAC	CTTCAACAGAGTCAGAATTAACACC
GCW_02760	ackA	GAAGCACCATTTCCTAAGTGAC	AGATCTATGAATTAGCCAAGCTC
GCW_01785	pgk	CTAAAAACAGTCCAGAACTAGG	TAAAGGTATTAACCATTCCCCC
GCW_03700	fba	GACCATGGTACTTATGAAGGTG	AAGTAGGGGGATAAATTCCGTG
GCW_03575	pgi	CCAGAAGTAAAGATAACCAAAGCC	ATTATTCCCAACATACTCCGTG
GCW_02725	pfkA	GTGGTGATGGTTCATATCAAGG	AGATCTACGATCTCTTGGGTTG
GCW_03285	gpmI	GAGATCACTATTCAAGACAATGACAG	ACACCACCATCAAAAAAGAAGG
GCW_02720	pykF	CCTTATTATGAAGTACCTTACTGGG	TCTGTCATTGGGAAATAGTGAG
GCW_00160	tktA1	AGAATACAAACAAAGCTCACGG	CACCTTCCCAAATAAATTACGG
GCW_03235	tktA2	AGATTACTGAAACGATCCTTGC	AAAGTTATCGATCTGATCTCGC
GCW_00795	rpiB	ATATTCGTAGCTGATCATACAGG	CAGTTATCATGTTGAATCGCAAG
GCW_03495	eutD	GAAGTTGATGGTGAAGTATGTGG	AACAGATTCACCTACACCTGAG
GCW_03255	prsA	CAGCATTTATTCTTCACTTCACC	CAGATTTACTAACCAAAGCAGG
GCW_02735	lpd	AACTTGATCATTAACTACCCGAC	CAATCATTAGTTGTGATCGGTG
GCW_03295	cpsG	ATCTTTACTGGTCAACATGGAG	GTAATAAGCAAAGATGATCCCC
GCW_02755	GCW_02755	AATACTGTGGTGGAATTGAAGG	TCAGTGTCAACTAAGTATCCCA
GCW_02490	GCW_02490	GAATCTAAGCAATCAGCATACGG	GAAATCACCTACATAATACGGCTTG
GCW_02495	GCW_02495	CGTGAGATAAAATTGAAGGTGC	TGTATAGGTGAGTGTCATCTGG
GCW_01270	hatA	ATGAGAACAGAATGTGAGATGG	GGTTTGTACCGATTTCTCTTCC
GCW_03695	rpoE	GGCTTCATTAATTGATAAGGCT	CCTTCATTGCTAATTTCAGGAGA
GCW_01075	spoT	TTATCATCTTCAGTAACCGCAC	GTGAAATGACATTCTTCTTGAGG
GCW_02645	GCW_02645	TATGACCAAAATAAGGAAGTTGCC	CTTAGAATCTGGTAACAAGTGGAAG
GCW_02635	GCW_02635	CAAGAGGTTAAAAGTGAGATCTGTG	GCTGTTGTTCTTCTAGTTCGTG
GCW_00440	GCW_00440	GTACAACCGATTTGATTCCAAG	AGTTCTTTTACGATATCACAGATCC
GCW_04025	hprK	AGAAGTATATGGTGAAGGGGTTC	GTTCGTTCGAAATTATAAGGTTGG
GCW_03690	GCW_03690	GATTAAGCTGGTTAAACGATCTCC	GTAACTTCTGAATCCCTTCAACC
GCW_03310	deoC2	AAGTTTATGAAGCCAAAACCTC	CTTATTTTGACTGGCTTGTTGC
GCW_03745	manA	TTCTACCCCATTTTGAATACCG	CTAGTGAGTTAAACTGTTTGGC
GCW_03735	fruA	TTCCAAACCACAATCAAACCAG	TTAATGTGAATGCGACTACACC
GCW_00765	ptsG1	TGACGAAAATGGACAAAGAGTG	ATAGATTGGATTAGTGAAGGGTGG
GCW_03100	ptsG2	CCTTTGGTATTCTAAGACTAGTAGC	GGTTAATCTGTTCAGATAAACTGC
GCW_04065	gyrA	ATGATGCTTCAGAACAAGAACC	CAATCTCAGATTTAGCCCGAAC
GCW_04070	gyrB	AATAACATTCACACCAACCAAGG	TAATCTTAGCAGCTTCTTCTGGA
GCW_02325	parC	AAACAACCCTAATCCCAAACC	CTTAAACTAATGCCAGGACCA
GCW_02330	parE	CATGACTTCCACCTTCAGAG	CATTTATTGCCAAAGACGGGA
GCW_03410	ruvA	TGCTTCAATCACGATCTCTG	GCACTGTTAACGATCAATGG
GCW_03405	ruvB	TTGTGGGTGATAACGAATGG	AGCCAGAATAAAGCATTAGACC
GCW_03920	ligA	CTTAGGGATGATCTCAGCAG	GTTACACCTCTAAGTTTCCCA
GCW_00470	uvrA	AAACCCTGCTTCACTTACTG	TTTCCACTACCAGAAACTCC
GCW_00205	uvrB	TGTGTTGTCGGAATTAACCT	ATCGCTTTAGTCATCTCATCAG
GCW_02010	uvrC	TATGAAGTGATCTATCGCAGGT	AGTGACGATCTTATCTGTTTGG

(continued on next page)

Table 2 (*continued*)

S6_ID	gene_name	for	rev
GCW_92149	uvrD	GTGTTGTAAGTTATGGTCCGA	CAGTGGTTTGACGATCATAGG
GCW_02695	recA	TGGTATGAGAATCAACTACCTG	GTAATCCCGAGGTTACAACTG
GCW_01370	mutM	GAACGTCATGTGTTAGTACGA	GCATAAATATTACCGATCCCAG
GCW_03930	msrA	AAAGCCGCTACGATACTGTG	TTATGCCAACTACAAACCAG
GCW_02085	nfo	ATTATGTTGTTCACGCTCCT	ATCAGCCATTGTTTCAAGAC
GCW_00810	hup1	AGGCGGAATTCTAGTTTGTG	ATTATCGCTGAATGTACTGGAG
GCW_02335	hup2	ATTTGTGCGAATCTACTGCA	AATGGCAGACGAAACTAACC
GCW_03435	ung	TGATTAATTCTTCCCAACCCAG	GTAATCATTGGTCAAGATCCGT
GCW_03245	umuC	CCAAGTCTAATGGTTCTATCGT	ATCGCTTGCAGATATCTCAC
GCW_00770	putA	CAAAAGCAGGTTATGAATATCGC	CTTTGATAATGGAGTGTTTAGGG
GCW_02665	potD	CAACTGCTTTATTAAACTCTGACTC	CATTAACCCCTTCAAGACCAAC
GCW_03110	spxA	TCATGTATTGGCTGTACGAGAG	CCATCAATCTTTTAGGAATCCCTG
GCW_93946	GCW_93946	GAATGTCTTCGTGTTAAGTGTC	AAAGCTAATGGGTTGATTGCTC
GCW_02245	mraW	TCGAGTTATCAACTAGATCGAG	GGAAATATCTTCTTGCAGGATG
GCW_03090	mscL	AACGTCATCTTAGTTTCAGCAC	CTTCAACACTTTGAGGTTCTGG
GCW_03085	GCW_03085	ATTATCTGTGTCCTTTCATCCG	CACTTACTTGAGCTAAGAAAGCAG
GCW_93751	GCW_93751	CAATAGTTTCAGGAAGACACGG	ACAGCATCACCATTATTTCCAG
GCW_02300	GCW_02300	TAAGTCTTCAGTTTGCTTCACG	TACTCTTCTAGTTGCTAGTTGTCC
GCW_92301	GCW_92301	TATCAAAGCCTTTCTAGCAACG	CACTATAAGTAGGTTTAGCTCAGG
GCW_01560	GCW_01560	AATCTAATCCAGGGACAGTTTC	GTGGCTACTAGAACATCATATCTAGG

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].

Acknowledgements

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