Transcriptomic analysis reveal differential gene expressions of *Escherichia coli* O157:H7 under ultrasonic stress

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**A B S T R A C T**

In order to uncover the molecular regulatory mechanisms underlying the phenotypes, the overall regulation of genes at the transcription level in *Escherichia coli* O157:H7 after ultrasonic stimulation were investigated by RNA-sequencing and real-time quantitative polymerase chain reaction. The results revealed that differential expressions of 1217 genes were significant when exposed at 6.67 W/mL power ultrasonic density for 25 min, including 621 up-regulated and 596 down-regulated genes. Gene transcription related to a series of crucial biomolecular processes were influenced by the ultrasonic stimulation, including carbohydrate metabolism, energy metabolism, membrane transport, signal transduction, transcription and translation. The most enriched pathways were further analyzed in each category. Specifically, genes encoded citrate cycle were down-regulated in *E. coli* O157: H7, indicating the capacity to decompose carbohydrate and produce energy were decreased under ultrasonic stress. Accompanied with energy loss, the membrane function was affected by the ultrasonic stimulation since the majority of genes encoded ATP-binding cassette transporters were down-regulated. Besides, the autoinducer 2-mediated signal transduction was also inhibited. The interesting thing, however, the protein translation processing was benefited under ultrasonic field. This phenomenon might due to the desperate need of stress response proteins when the bacteria were under stress. We believed that the sonomechanical and sonochemical effects generated by acoustic cavitation were responsible for those gene expression changes.

**1. Introduction**

As an effective non-thermal processing method, ultrasound can achieve the microbial safety and assure the food quality at the same time [1,2]. In most studies, acoustic cavitation is widely accepted as the inactivation mechanism, and its mode of action on microorganisms has been in the focus of research for many years [3–5]. Previous studies have shown the following multiple lethal targets of ultrasound at the cellular level. The shear forces in the cavitation field acted from the outside, eventually causing the break down of cell wall and irreversible cell membrane damage [6], and the effects of microstreams on intracellular structures were also observed via transmission electron microscopy and fluorescent microscope [7]. Besides, the chemical effects were considered to be the reason for the damage of nucleic acid [8]. In addition to ultrasonic disruption, ultrasonic field was found to trigger genetically encoded apoptosis in *E. coli* O157:H7, resulting in the exhibition of apoptotic biochemical hallmarks like exposed phosphatidylserine and activated caspases [9]. From the above, we can conclude that the effects of ultrasound on cell structures and compounds have been well illustrated, while the actual mode of action of ultrasound at the molecular level is mainly unstudied. But this knowledge is essential to uncover the underlying causes of phenotypic changes and for the safe application of ultrasound devices on microbial inactivation.

More recently, investigations have been expanded to the molecular level of non-thermal processing technologies on microorganisms. It was found that plasma treatment could induce gene expression changes in *Staphylococcus aureus* at transcription level by the quantitative real-time polymerase chain reaction (qPCR), resulting in the activation of oxidative stress responses and DNA damage repair systems to counter the stimulus [10]. Further, transcriptomic analyses were applied as powerful tools to uncover global changes in bacterial gene expression. Among them, microarrays have been widely used to investigate the transcriptional responses of bacteria after exposure to stresses, including UV light [11], plasma [12], sunlight [13], high pressure [14]. Next-
generation high-throughput sequencing technology, RNA-Sequencing, can provide higher sensitivity, higher resolution and wider detection range than microarray methods. This promising technique quantifies relatively low abundance and provides more in-depth information to uncover the complex gene regulatory networks associated with stress response. Amrani et al. [15] used RNA-seq to study the response of deep-sea piezophilic bacteria; the transcriptomics identified 65 differentially expressed genes that were mainly distributed in aromatic amino acid and glutamate metabolisms, energy metabolism, signal transduction, and unknown function. Al-Jassim et al. [16] found that although solar irradiation remained effective in reducing the bacteria number, transcriptomics revealed the overall upregulation of regulatory, repair and protective mechanisms of E. coli strains. Therefore, we could see that viable bacteria remained and were able to express genes that enable survival despite the external pressures, raising concerns regarding risks of the incomplete sterilization. However, transcriptome-level studies of gene expression patterns to diverse ultrasonic conditions and the downstream regulatory networks have not been reported.

In this study, foodborne pathogen E. coli O157:H7 was selected as the model microbe. A comprehensive and detailed understanding at the transcription level in E. coli O157:H7 after ultrasound treatment was investigated by RNA-sequencing. The most enriched pathways were further analyzed in each category in order to reveal the in-depth relationships between the gene expression levels and phenotypic changes. The results of this study would give us insights into stress response mechanisms and help enable the design of more effective and safe ultrasonic devices.

2. Materials and methods

2.1. Bacteria preparation

Escherichia coli O157:H7 NCTC 12,900 was used in this study, which was obtained from China Center of Industrial Culture Collection. The cultures were kept at −80 °C in nutrient broth (NB; Hope Bio, Qingdao, China) with 50% glycerol. The strain was incubated in NB at 37°C with shaking at 150 rpm to reach the stationary phase (∼10^9 CFU/mL). Bacterial cells were collected by centrifugation at 2,320 g for 10 min and washed twice with 0.85% (w/v) sterile saline solution.

2.2. Ultrasonic exposure

The experiments were performed using an ultrasonic processor with 10-mm-diameter probe (JY92-IIDN, Scientz, Ningbo, Zhejiang, China). Thirty milliliters of cell suspension were placed into cylindrical tube and sonicated by positioning the probe into the sample for 2 cm. The operating frequency of this ultrasonic processor is 20 kHz and the total input power is 900 W. Power density (D, W/mL) of ultrasound dissipated into the medium with volume V is given by $D = P/V$, where P is the input power. Power intensity (I, W/cm^2) dissipated from a probe tip with radius r is given by $I = P/(N_r^2)$. The glass tubes containing the bacterial suspension were immersed in a low-temperature thermostatic water bath to maintain temperature at 20°C in order to prevent a lethal thermal effect during the sonication treatment. In that case, the heat generated in the sonication phase is normally radiated into the water bath, which can eliminate the influence of the thermal effect. In a previous study, we did a series of research about ultrasound-induced E. coli O157:H7 cell death using defined power density, power intensity, and duration times. It turned out that when operating at 200 W power, 6.67 W/mL power density and 255 W/cm^2 power intensity for 25 min, multiple lethal targets of ultrasound on E. coli O157:H7 at the cellular level were achieved [9]. Therefore, we chose this parameter to further investigate the transcriptomic changes to uncover the the molecular regulatory mechanisms that underlie the phenotypes.

2.3. Total RNA isolation

For each biological sample, total RNA was isolated separately using the TRIZOL reagent (Invitrogen, Camarillo, CA, USA) following instructions from the manufacturer. The quantity and quality of RNA was assessed by Nanodrop (Thermo Scientific, Sugarland, TX, USA) and Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The OD260/280 was between 2.0 and 2.2 and RNA 23S/16S was between 1.7 and 1.9. The RNA integrity number was above nine, considering to be undegraded.

2.4. cDNA library construction and sequencing

The cDNA library construction and sequencing were performed at the Beijing Genomics Institute (BGI-Shenzhen, Shenzhen, China). Briefly, the mRNA was fragmented into small pieces using fragmentation buffer. The first-strand cDNA was synthesized using mRNA template and SuperScript II Reverse Transcriptase kit (Invitrogen, Camarillo, CA, USA). The second-strand cDNA was then synthesized using SuperScript Double-Stranded cDNA Synthesis kit (Invitrogen, Camarillo, CA, USA). Then agarose gel electrophoresis was performed to select suitable fragment size for PCR amplification after end reparation, poly (A) addition and adapters ligation. The final cDNA library was sequenced using an Illumina HiSeq Xten platform, producing 150 bp paired-end reads.

2.5. Bioinformatic data analysis

To obtain clean reads data, raw reads data were filtered by removing low-quality reads, reads containing adapters and ambiguous “N” bases greater than 5%. The Bowtie2 software was used to map the high-quality clean reads. Then RSEM software was used to assess the gene expression level and further normalized by the FPKM method. Differential expression analysis was performed using the DESeq2, and false discovery rate (FDR) was applied as the p-value threshold. Then fold changes between treated and control group were calculated after comparing the FPKM values. A p-value smaller than 0.05 with fold changes ratio larger than 2 were set as the threshold for differentially expressed genes (DEGs). The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the DEGs were further conducted.

2.6. Quantitative real-time PCR validation

Representative genes were analyzed using Thermofisher 7300Plus Real-Time PCR System to evaluate the validity of Illumina analysis.

| Genes | Primer sequence(5′ → 3′) |
|-------|--------------------------|
| acnA  | CGATCTCCTATGAAACCAGCA    |
| oppF  | CGATAGGCTCAGAAGGCGG      |
| cysC  | TGGCCATCATCCTCTGTTG      |
| lonR  | TGCCCTCGGGTTTAAAGG       |
| rpsL  | GTGGTTATCTCTGTCGGGC       |

Table 1 | Amplification primers of target genes.
Total RNA was reverse-transcribed into cDNA using 5X All-in-One RT MasterMix (Applied Biological Materials Inc., Vancouver, Canada). Then PCR reactions were carried out using EvaGreen qPCR Mastermix (Applied Biological Materials Inc., Vancouver, Canada) with the following programs: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 63°C for 30 s. The relative expression level of target genes was measured with the $2^{-\Delta\Delta Ct}$ method and 16sRNA was used as the reference gene. All tests were performed at least three times. The primers are listed in Table 1.

3. Results

3.1. Distribution of DEGs

Two groups of *E. coli* O157:H7 cells were prepared for Illumina transcriptome sequencing. One was treated by ultrasound with optimal conditions and the other was untreated as a control group. A total of 4682 genes was identified by RNA-seq, of which 1217 was significantly differentially expressed, including 621 up-regulated genes and 596 down-regulated genes. The volcano of MA plot (Fig. 1) depicted the M ($\log_2$ FoldChange) against A ($\log_2$ RPKM/expression level) for all DEGs between two groups. The red points and blue points marked out up-regulated and down-regulated DEGs with significant difference ($P_{adj} \leq 0.05$), respectively. From the clustering heat map (Fig. 2) we could see the gene expression levels from a global perspective. Different colors revealed the differences of expression levels for all DEGs, showing varying degrees of over-expression (red) and under-expression (blue).

And the more close the color distribution of two samples was, the more similar the expression patterns of two samples were.

3.2. GO enrichment analysis of DEGs

To obtain an overview of the changes of gene functions after ultrasound treatment, the DEGs were annotated by blasting in GO database. Fig. 3 showed three GO lists ranked according to their enrichment scores, including information on biological processes, cellular components and molecular functions. In the ontology of biological processes, most DEGs were mainly gathered at the term ‘metabolic process’, ‘cellular process’, ‘single-organism process’, ‘localization’, ‘biological regulation’. For the cellular component ontology, the main categories were ‘cell’, ‘cell part’, ‘membrane’, ‘macromolecular complex’. Besides, ‘catalytic activity’, ‘binding’, ‘transporter activity’ were the three main distributed terms in the molecular function ontology.

3.3. Pathway enrichment analysis of DEGs

Pathway enrichment analysis based on KEGG database were performed to reveal metabolic and signal transduction pathways of DEGs. KEGG Pathway is mainly divided into the following five categories: cellular processes, environmental information processing, genetic information processing, metabolism and organismal systems. Fig. 4 showed the most enriched pathways of DEGs were metabolism, environmental information processing and genetic information processing. In the metabolism category, the DEGs were mainly distributed in the
carbohydrate metabolism', 'amino acid metabolism', 'energy metabolism', 'metabolism of cofactors and vitamins', 'nucleotide metabolism' and 'lipid metabolism' terms. Notably, 73 DEGs were annotated to 'membrane transport' and 33 DEGs were annotated 'signal transduction' in the category of environmental information processing. As for genetic information processing category, three most enriched terms 'translation', 'replication and repair', 'folding, sorting and degradation' were worthy of attention. In addition, a scatter plot for the top 20 of KEGG enrichment results were generated as Fig. 5. Greater rich factor means greater intensiveness of DEGs in this pathway term, and larger dot means larger numbers of DEGs. The color represents the Q-value: the bluer the color is, the smaller the value is, and the more obvious the enrichment result will be. It revealed the most enriched pathways were 'ribosome', 'metabolic pathways', 'oxidative phosphorylation', 'quorum sensing', 'carbon metabolism', 'tricarboxylic acid cycle'. From above, we could understand the functional distribution of DEGs at the macro level and further predict the potential molecular regulations under ultrasonic stress.

3.4. Quantitative real-time PCR validation

In order to validate the reliability of transcriptomic data by RNA-seq, five DEGs were selected from different pathways (acnA, oppF, cyoC, lpxR, rpsI) for qRT-PCR analysis. In general, the qRT-PCR results (Table 2) showed accordance with the sequencing results, indicating that the transcriptome data were reliable.

4. Discussion

The above results showed the gene regulation at the transcription level in E. coli O157:H7 after ultrasound treatment, and the most enriched pathways related to crucial cellular processes were further analyzed in each category (Table 3). The main metabolic regulations of E. coli O157:H7 cells under ultrasonic stress were illustrated in Fig. 6, including carbohydrate and energy metabolism, membrane transport function, transcription and translation function, and quorum sensing system.

4.1. Carbohydrate and energy metabolism

The citrate cycle, also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle, is a hub of energy metabolism and connects carbohydrate, protein and fat metabolism [17]. In bacterial cells, the TCA reactions are performed in the cytosol, and carried out by a series of enzymes that oxidize acetyl-CoA completely into carbon dioxide with the release of energy. The cycle also converts NAD$^+$ into NADH, FAD into FADH$_2$, and produces one GTP. The NADH and FADH$_2$ will be fed into the oxidative phosphorylation pathway to produce rich energy in the form of ATP [18]. Compared to the control group, the DEGs involved in TCA cycle were down-regulated in ultrasound-treated E. coli O157:H7 cells, including genes encoded aconitate hydratase (acnA, acnB), 2-oxoglutarate dehydrogenase (sucA, sucB), succinyl-CoA synthetase (sucC, sucD), succinate dehydrogenase (sdhA, sdhB), fumarate hydratase (fumA, fumC), malate dehydrogenase (mdh). This meant the overall yields of
NADH and FADH$_2$ in *E. coli* O157:H7 cells might be reduced after ultrasound treatment. Meanwhile, the gene expression related of NADH dehydrogenase (*nuoN, nuoL, nuoM, nuoH*), succinate dehydrogenase (*sdhA, sdhB*), and cytochrome c oxidase (*cyoA, cyoB, cyoC*) were also down-regulated significantly, resulting in the inefficiencies of oxidative phosphorylation. We concluded that the accumulation of proton (H$^+$) in the electron transport chain might be decreased when *E. coli* O157:H7 cells were treated by ultrasound. Nevertheless, the ATPase subunits related DEGs were up-regulated under the ultrasonic stress. As we know, ATP is synthesized by ATP synthase using energy stored in the proton gradient across the plasma membrane in bacterial cells [19]. The up-expression of ATP synthase might be the coping measure to encounter the reduced accumulation of proton gradient, but it couldn’t reverse the general downward trend in ATP production. This is consistent with our previous study, which showed the ATP contents decreased after ultrasound treatments [9]. The affected energy metabolism might damage the metabolic capacity and normal physiological function of bacteria.

### 4.2. Membrane transport function

ATP-binding cassette (ABC) transporters are a superfamily of membrane-associated bacterial proteins, comprising two transmembrane domains (TMDs) and two nucleotide binding domains (NBDs) [20,21]. The NBDs can bind and hydrolyze ATP, and further transmit conformational changes to TMDs, opening the conduit between the TMDs to transport diverse substrates across the membranes [22]. In *E. coli* cells, ABC transporters constitute the largest protein family, including approximately 80 distinct systems which represent 5% of the total genome [23]. Our results showed that some DEGs related to ABC transporters were down-regulated after exposed to ultrasound, such as genes involved in zinc transport system (*znuB, znuC*), oligopeptide transport system (*oppB, oppC, oppF*), histidine transport system (*hisQ, hisI, hisM, hisP*), lysine / arginine / ornithine transport system (*argT*), lipoprotein-releasing and transport system (*lolC, lolD, lolE*). As we can see, those transporters listed above were characterized by the extracellular binding protein which facilitated the substrates into the cell, referred as prokaryotic-type importers [24]. Therefore, the down-regulation of genes coded ABC importers might affect the membrane transport function under ultrasonic stress, resulting in the suppression of uptake of nutrients and other molecules. This might be the reason that the membrane permeability and ion exchanges across membrane were changed after ultrasound treatments [9].

### 4.3. Signal transduction function

Quorum sensing (QS) is a kind of cell-to-cell communication system, in which bacteria monitor the density of the cell population by measuring the concentration of signal molecules and further regulate the gene expression accordingly [25,26]. The signal molecules, termed as autoinducers, are produced and secreted into the extracellular environment and gradually increase in concentration as the population of QS bacteria grows [27,28]. Our data showed that after ultrasound treatment, the genes related to autoinducer 2 (AI-2) mediated QS system (*lsrB, lsrC, lsrD, lsrK, lsrF, lsrR*) were down-regulated. AI-2 is involved in the only shared QS mechanism by both Gram-negative and Gram-positive bacteria [29]. In the periplasmic space, the AI-2 signal molecule binds to LsrB, and then passes through the heterodimeric transmembrane channel formed by LsrC and LsrD, accompanied with the hydrolysis of ATP by LsrA to provide energy for signal molecule transport [30]. AI-2 is phosphorylated by LsrK after entering the cytoplasm, the phosphorylated AI-2 is processed by LsrF and LsrG, and then binds to the repressor protein LsrR. The conformation of LsrR is changed to
release its inhibitory effect, thereby initiating the transcription of the *lsr* operon [31]. However, the transcription level of the *lsr* operon was reduced and quorum sensing was suppressed under the ultrasonic stress. This process might affect the expression of genes crucial for virulence and survival, decreasing the ability to communicate between cells.

### 4.4. Transcription and translation function

For prokaryotic cells, regulation of gene transcription plays a crucial role in responding to environmental changes and adapting metabolic processes to new conditions. The RNA polymerase of *E. coli* consists of a multisubunit core enzyme (αββ′w) and one of seven species of subunit σ. The σ factors are important for RNA polymerase to recognize promoters and initiate transcription [32,33]. After transcription starts, the σ factors can unbind and let the core enzyme transcribe RNA. We could see that the gene expressions of α, β, β′ (rpoA, rpoB, rpoC) were up-regulated after ultrasound treatment, indicating the function of transcribing RNA might be strengthened under stress condition. It was also reported that genome transcription pattern could be altered through modulating the gene selectivity of *E. coli* RNA polymerase by interacting with hundreds of transcription factor species [34]. Besides, after the ultrasound treatment, DEGs related to large ribosomal proteins and DEGs about small ribosomal proteins are up-regulated compared to the normal cells. The ribosomes are served as the sites of protein synthesis. Prokaryotic ribosomes have the sedimentation rate of 70S, and are made of a large (50S) and a small (30S) subunit [35]. In *E. coli*, the large subunit contains a 5S rRNA (120 nt) subunit, a 23S rRNA (2,904 nt) subunit bound to 34 different proteins. The small one is composed of a 16S rRNA (1,542 nt) subunit and 21 ribosomal proteins [36]. It seemed like the expression of genes encoded above subunits was up-regulated, indicating the synthesis ability of ribosomes increased in the face of these environmental stresses, which might improve the translation function to some extent. In fact, that has been proved that a series of stress response proteins were accumulated after ultrasound treatments [37].

### 5. Conclusion

The results of this study suggested that gene transcription related to a series of crucial cellular processes was down-regulated under ultrasonic stress. Among them, the down-regulation of genes encoded carbohydrate catabolism and respiratory chain indicated the capacity to decompose carbohydrate and produce energy was decreased, resulting in the decrease of ATP production at the phenotypic level. Besides, genes involved in ABC transporters were also down-regulated, which inhibited the transmembrane transport function and changed the membrane permeability. However, ribosome synthesis and assembly maturation were shown to be up-regulated, indicating the synthesis capacity of some proteins had increased under ultrasonic stress. From this study, we
have elucidated the relationship between some biological characteristics and gene regulations. In the future, the mutants of *E. coli* O157:H7 will be constructed to verify the functions of differentially expressed genes for the further identification of the key regulatory network.

**CRediT authorship contribution statement**

**Jiao Li:** Investigation, Project administration, Writing - original draft, Funding acquisition. **Donghong Liu:** Conceptualization, Supervision, Funding acquisition. **Tian Ding:** Investigation, Writing - review & editing, Supervision, Funding acquisition.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Table 2

The qPCR verification of differentially expressed genes of *E. coli* O157:H7.

| Genes | Description                     | qPCR     | RNA-seq   |
|-------|---------------------------------|----------|-----------|
| acnA  | aconitate hydratase             | −1.8 down| −3.1 down |
| oppF  | oligopeptide transport system ATP-binding protein | −1.1 down | −1.3 down |
| cyoC  | cytochrome *c* ubiquinol oxidase subunit III | −1.2 down | −3.1 down |
| lpxR  | lipid A 3-O-deacylase            | 1.5 up   | 3.4 up    |
| rpsI  | small subunit ribosomal protein S9 | 1.4 up   | 2.6 up    |

![Fig. 5. Pathway enrichment map of differentially expressed genes.](image)
Table 3
Major metabolic pathways involved in differentially expressed genes.

| Gene ID   | Genes    | Ratio | Description                                      |
|-----------|----------|-------|--------------------------------------------------|
| TC cycle  | acnA     | −3.1  |aconitate hydratase                              |
|           | acnB     | −1.5  |aconitate hydratase 2                            |
|           | sucA     | −2.4  |2-oxoglutarate dehydrogenase E1 component        |
|           | sucB     | −2.2  |2-oxoglutarate dehydrogenase E2 component        |
|           | sucC     | −2.5  |succinyl-CoA synthetase beta subunit             |
|           | sucD     | −2.8  |succinyl-CoA synthetase alpha subunit            |
|           | sdhA     | −2.4  |succinate dehydrogenase, flavoprotein subunit    |
|           | sdhB     | −2.2  |succinate dehydrogenase, iron-sulfur subunit     |
|           | sfoA     | −2.3  |fumarate hydratase, class I                      |
|           | sfoC     | −2.8  |fumarate hydratase, class II                     |
|           | mdh      | −1.0  |malate dehydrogenase                             |
|           | SdhD     | −1.9  |succinate dehydrogenase, membrane anchor subunit |
| Oxidation respiratory chain | mnuN | −2.8  |NADH-quinone oxidoreductase subunit N        |
|           | mnuL     | −2.2  |NADH-quinone oxidoreductase subunit L             |
|           | mnuM     | −2.0  |NADH-quinone oxidoreductase subunit M             |
|           | mnuH     | −2.2  |NADH-quinone oxidoreductase subunit H             |
|           | mnuI     | −2.1  |NADH-quinone oxidoreductase subunit J             |
|           | mnuJ     | −1.9  |NADH-quinone oxidoreductase subunit I             |
|           | frdA      | −1.5  |fumarate reductase flavoprotein subunit          |
|           | frdB     | −1.3  |fumarate reductase iron-sulfur subunit           |
|           | sfoA     | −2.4  |succinate dehydrogenase flavoprotein subunit     |
|           | sfoB     | −2.2  |succinate dehydrogenase, iron-sulfur subunit     |
|           | sfoD     | −1.9  |succinate dehydrogenase, membrane anchor subunit |
| ABC transporters | mnuA | −2.3  |cytochrome o ubiquinol oxidase subunit I         |
|           | mnuB     | −1.6  |cytochrome bd ubiquinol oxidase subunit II       |
|           | mnuC     | −1.8  |cytochrome o ubiquinol oxidase subunit II        |
|           | mnuD     | −3.1  |cytochrome o ubiquinol oxidase subunit III       |
|           | aprF     | 1.6   |F-type H + -transporting ATPase subunit b        |
|           | aprA     | 1.2   |F-type H + -transporting ATPase subunit alpha    |
|           | aprB     | 1.1   |F-type H + -transporting ATPase subunit a        |
|           | aprH     | 1.5   |F-type H + -transporting ATPase subunit delta    |
| Quorum sensing | mshB | −1.1  |zinc transport system permease protein           |
|           | mshC     | −1.1  |zinc transport system ATP-binding protein        |
|           | hisQ     | −1.3  |histidine transport system permease protein      |
|           | hisR     | −1.1  |histidine transport system ATP-binding protein   |
|           | hisM     | −1.5  |histidine transport system permease protein      |
|           | hisP     | −1.7  |histidine transport system ATP-binding protein   |
|           | loIC     | −1.2  |lipoprotein-releasing system protein LoIC         |
|           | loID     | −1.2  |lipoprotein-releasing system ATP-binding protein |
|           | loIL     | −1.2  |lipoprotein transporter subunit LoIL             |
|           | oppB     | −1.3  |oligopeptide transport system permease protein   |
|           | oppA     | −1.3  |oligopeptide transport system permease protein   |
|           | oppF     | −1.3  |oligopeptide transport system ATP-binding protein|
|           | argF     | −1.5  |lysine/arginine/ornithine transport system       |
|           | argT     | −1.5  |substrate-binding protein                        |
| Transcription and translation | rpoA | 2.1   |DNA-directed RNA polymerase subunit alpha        |
|           | rpoB     | 2.0   |DNA-directed RNA polymerase subunit beta         |
|           | rpoC     | 1.7   |DNA-directed RNA polymerase subunit beta’        |
|           | groA     | 2.0   |transcription elongation factor GreA             |
|           | rapU     | 2.0   |large subunit ribosomal protein L16              |
|           | rpsM     | 1.8   |small subunit ribosomal protein S13              |
|           | rpsD     | 1.9   |small subunit ribosomal protein S4               |
|           | rpsB     | 2.4   |large subunit ribosomal protein L13              |
|           | rpsQ     | 2.3   |small subunit ribosomal protein S17              |
|           | rpsK     | 1.9   |small subunit ribosomal protein S11              |
|           | rpsG     | 2.1   |large subunit ribosomal protein L17              |
|           | rplJ     | 2.6   |small subunit ribosomal protein S9               |
|           | rimM     | 1.3   |ribosome maturation factor RimM                   |
|           | rimP     | 2.1   |ribosome maturation factor RimP                   |
Fig. 6. Schematic diagram of main metabolic regulations of E. coli O157:H7 cells under ultrasonic stress. (arrows indicate up-regulation or down-regulation of differentially expressed genes on related pathways).

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