Current Research in Food Science 5 (2022) 798–806

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Research Paper

Global transcriptomic analysis of ethanol tolerance response in *Salmonella* Enteritidis

Shoukui He [a], Yan Cui [a], Rui Dong [a], Jiang Chang [a], Hua Cai [b], Hong Liu [b], Xianming Shi [a,*]

[a] *MOST-USDA Joint Research Center for Food Safety, School of Agriculture and Biology, State Key Lab of Microbial Metabolism, Shanghai Jiao Tong University, Shanghai, 200240, China*
[b] *Shanghai Municipal Center for Disease Control and Prevention, Shanghai, 200050, China*

ARTICLE INFO

Handling editor: Siyun Wang

Keywords:
Salmonella enteritidis
Ethanol tolerance
Transcriptomic analysis
Gene expression
Adaptation pathway

ABSTRACT

Adaptation to sublethal amounts of ethanol enables *Salmonella* Enteritidis to survive under normally lethal ethanol conditions, which is referred to as the ethanol tolerance response (ETR). To uncover mechanisms underlying this adaptative response, RNA-seq and RT-qPCR techniques were employed to reveal global gene expression patterns in *S.* Enteritidis after sublethal ethanol treatment. It was observed that 811 genes were significantly differentially expressed in ethanol-treated cells compared with control cells, among which 328 were up-regulated and 483 were down-regulated. Functional analysis revealed that these genes were enriched in different pathways, including signal transduction, membrane transport, metabolism, transcription, translation, and cell motility. Specifically, a couple of genes encoding histidine kinases and response regulators in two-component systems were up-regulated to activate sensing and signaling pathways. Membrane function was also influenced by ethanol treatment since ABC transporter genes for transport of glutamate, phosphate, 2-aminoethylphosphonate, and osmoprotectant were up-regulated, while those for transport of iron complex, manganese, and ribose were down-regulated. Accompanied with this, diverse gene expression alterations related to the metabolism of amino acids, carbohydrates, vitamins, and nucleotides were observed, which suggested nutritional requirements for *S.* Enteritidis to mount the ETR. Furthermore, genes associated with ribosomal units, bacterial chemotaxis, and flagellar assembly were generally repressed as a possible energy conservation strategy. Taken together, this transcriptomic study indicates that *S.* Enteritidis employs multiple genes and adaptation pathways to develop the ETR.

1. Introduction

Ethanol has long been employed for chemical disinfection, food preservation, and colorant dissolution in food industries. In food processing plants, ethanol can be utilized for the disinfection of food processing tools, conveyor belts, and food contact surfaces (Dev Kumar et al., 2020; Fagerlund et al., 2017; Shen et al., 2016). In addition, direct addition of ethanol (0.5–5%) is beneficial for prolonging shelf life of foods (Doulia et al., 2000; Katsinis et al., 2008; Shibasaki, 1982), while immersion in ethanol (2.5–70%) is effective in controlling postharvest decay of fruits (Dao and Dantigny, 2011). Ethanol is also a common component in fermented beverages, fruit products and other foods at major or minor levels (He et al., 2021a). Therefore, there exist opportunities for pathogenic bacteria to adapt to sublethal concentrations of ethanol during food processing.

Adaptation to sublethal levels of ethanol is able to enhance bacterial tolerance to subsequent lethal ethanol challenges, which is termed the ethanol tolerance response (ETR) (He et al., 2016). This adaptive response has been observed in a number of pathogenic bacteria such as *Salmonella* Enteritidis, *Cronobacter sakazakii*, *Bacillus cereus*, *Vibrio parahaemolyticus*, and *Listeria monocytogenes* (Browne and Dowds, 2001, 2002; Chiang et al., 2006; He et al., 2021b; Huang et al., 2013; Lou and Yousef, 1997). For example, exposure to a sublethal level of 5% ethanol has been demonstrated to induce bacterial tolerance to 15% ethanol challenge in *S.* Enteritidis (He et al., 2016). The development of ETR in pathogenic bacteria represents a concern to food safety since it may counteract the effectiveness of currently employed food control measures (He et al., 2021a). Therefore, it is crucial to uncover why pathogenic bacteria mount ethanol tolerance.

Physiological and proteomic approaches have been employed to

* Corresponding author.
E-mail address: xmshi@sjtu.edu.cn (X. Shi).

https://doi.org/10.1016/j.crfs.2022.04.011
Received 31 December 2021; Received in revised form 7 April 2022; Accepted 26 April 2022
Available online 6 May 2022
2665-9271/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

explore the ETR mechanisms in pathogenic bacteria. Physiological analysis revealed that cell membrane permeability and fatty acid composition were involved in the ETR of *V. parahaemolyticus* (Chiang et al., 2006, 2008). By means of the 2-DE technique, Yeh (2012) found that a total of 16 proteins were differentially expressed after exposure of *C. sakazakii* to 5% ethanol for 60 min. Moreover, the same sublethal ethanol treatment resulted in the differential expression of 138 proteins belonging to metabolism, enterobactin biosynthesis, virulence, and other pathways in *S.* Enteritidis (He et al., 2019). To our best knowledge, however, there is no available literature on elucidating the ETR mechanisms in pathogenic bacteria at the transcriptome level.

RNA-seq-based transcriptomics is a powerful tool for exploring stress response mechanisms in pathogenic bacteria (Lamas et al., 2019). This approach has been extensively used to characterize bacterial response to food processing-related stress factors such as acid, low temperature, erythorbyl laurate, and acidified sodium chlorite (Hingston et al., 2017; Hu et al., 2018; Park et al., 2019; Weerasooriya et al., 2021; Zhou et al., 2020). In addition, RNA-seq technology was successfully applied in revealing genes and pathways responsible for the survival of *Salmonella enterica* in different foods, including peanut oil, powdered milk, milk chocolate, black pepper, and egg white (Crucello et al., 2019; Deng et al., 2012; Huang et al., 2019). It is thus expected that RNA-seq will be helpful in elucidating the ETR mechanisms in foodborne pathogens.

In our previous work, *S.* Enteritidis was found to mount the ETR upon adaptation to a sublethal level (5%) of ethanol (He et al., 2016; 2021b). The current work aimed to unravel mechanisms of ETR in this pathogen by RNA-seq analysis, which might be useful in designing effective food control measures.

2. Materials and methods

2.1. Bacterial strains

S. Enteritidis ATCC 13076 was stocked at −80 °C in Luria-Bertani broth (LB) (Oxoid, Hampshire, UK) supplemented with 50% glycerol (Aladdin, Shanghai, China). Prior to each test, this bacterium was resuscitated by two successive transfers in 5 mL LB broth at 37 °C for 24 h. An aliquot (500 μL) of activated cultures was then inoculated into 50 mL LB broth, followed by incubation at 37 °C/200 rpm for 5 h to reach the late log phase (He et al., 2016).

2.2. Ethanol treatments

Sublethal ethanol treatment was carried out by exposure of *S.* Enteritidis to 5% ethanol for 60 min, which was previously identified as an optimal condition to induce bacterial ETR (He et al., 2016). Briefly, 1 mL of late-log-phase culture was centrifugated at 8000 *g* for 10 min and resuspended in 10 mL fresh LB broth with or without 5% ethanol (Changshu Yangyuan Chemical Co. Ltd., Jiangsu, China), respectively. Subsequently, these samples were incubated at 25 °C/170 rpm for 60 min to produce ethanol-treated and control cultures for RNA sequencing.

2.3. Total RNA isolation

Total RNA was extracted from ethanol-treated and control cultures of *S.* Enteritidis using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The Nanodrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA) and Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) were then utilized to verify RNA quantity and quality. The RNA integrity number was between 9.7 and 9.9, indicating that RNA was undegraded.

2.4. cDNA library construction and RNA sequencing

The cDNA library was constructed and sequenced at BGI Group (Shenzhen, Guangdong, China). Briefly, specific biotinylated oligonucleotides were used to remove rRNA from total RNA. The Illumina TruSeq Stranded Kit (Illumina, Inc., USA) was then utilized to construct a strand-specific cDNA library. The resulting cDNA library was sequenced using the Illumina HiSeq 4000 platform (Illumina, Inc., USA). The quality of sequencing data was evaluated after removing the adaptors by the SOAP software (v2.21) with optimized parameters (-m 0-x 1000-s 28-l 32-v 5-r 1-p 3). Subsequently, the HISAT software (v2.0.1-beta) was used to map high-quality reads to the genome of *S.* Enteritidis str. P125109. The relative expression level of each gene in ethanol-treated samples compared with control samples was calculated by the FPKM method using the RSEM (v1.2.12) and Bowtie2 (v2.2.5) softwares. Differential gene expression analysis was carried out based on the DESeq2 method. The criteria for the selection of significant gene expression were set as follows: fold change ≥2 and *P*-value (P_{adj}) ≤ 0.05. Raw sequences have been deposited in the National Microbiology Data Center (NMDC) database under BioProject number NMDC10018093.

2.5. Bioinformatic analysis of differentially expressed genes

Differentially expressed genes were mapped to the items in Gene Ontology (GO) database (http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/pathway.html) to identify their biological functions and molecular pathways. A Q-value ≤ 0.05 was utilized to recognize significantly enriched GO terms and KEGG pathways.

2.6. Quantitative real-time PCR (RT-qPCR) analysis

Total RNA was extracted from ethanol-treated and control cells of *S.* Enteritidis by the Trizol reagent (Invitrogen, Carlsbad, CA, USA), followed by reversed transcription into cDNA by PrimeScript™ RT reagent kits with gDNA Eraser (TaKaRa, Dalian, China). Primers were designed by the software Primer 5 and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Subsequently, PCR reactions were carried out using primers listed in Table 1 with the following programs: 1 cycle at 95 °C for 5 min, and 40 cycles at 95 °C, 55 °C, and 68 °C for 5 s, 15 s and 30 s, respectively. Relative gene expression levels in ethanol-treated samples compared with control samples were calculated by the $2^{-\Delta\Delta Ct}$ method with 16 S rRNA as the reference gene (Livak and Schmittgen, 2001).

2.7. Statistical analysis

RNA sequencing was carried out with three biological replicates. RT-qPCR test was performed in duplicate, and the resulting data were presented as mean ± standard deviation. Statistical comparison of gene expression levels in RT-qPCR tests was determined by Student's t-test ($P < 0.05$).

3. Results and discussion

3.1. Identification of differentially expressed genes

In the current work, *S.* Enteritidis was adapted with 5% ethanol for 60 min, which was previously confirmed as an optimal sublethal

Table 1
Primers used for RT-qPCR analysis.

Gene	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
asr	CTCTTCCGCAGCATTCGC	GCCGGTTGAGTAGTTGGTTT
srlE	ATCACCGAGCAGAGCG	ACAGCAGCGGGAAAGA
rbfA	AAAAGCGGGCATCAAA	ACGCATCCCTTCCACC
SEN1805	TGATTGTAAGAGCGGTAA	CGCTTCTGTTTCGTGT
SEN1383	GGCTGGCGAATGGTGA	AGCGAGCATGTTCTGGAAAG
16 S rRNA	CAGAAGAAGCACCGGCTAAC	GACTCAAGCCTGCCAGTTTC

treatment that induced the highest magnitude of ETR (He et al., 2016). Ethanol-treated and control cells were then subjected to RNA-seq using the high-throughput Illumina sequencing platform. A large number of raw reads were generated and after rigorous data filtration, an average of 14,436,838 and 14,418,735 clean reads were collected from ethanol-treated and control groups, respectively. A total of 98.2% of clean reads were mapped to the reference genome for both groups. These data suggested that RNA-seq quality was confidential for further analysis.

Gene expression levels were then compared between ethanol-treated and control samples. The global transcript profile of *S.* Enteritidis in the ethanol-treated group compared with the control group was shown in Fig. 1. According to the cutoff criteria of fold-change ≥ 2 and $P_{adj} \leq 0.05$, a total of 811 genes were significantly differentially expressed in *S.* Enteritidis in response to sublethal ethanol treatment, of which 328 were up-regulated and 483 were down-regulated. Information on these differentially expressed genes was provided in Supplementary Table 1.

3.2. Functional analysis of differentially expressed genes

Differentially expressed genes in *S.* Enteritidis under sublethal ethanol stress were annotated to three GO categories (Fig. 2). In the biological process category, a large number of differentially expressed genes were related to cellular processes, metabolic processes, localization, and biological regulation. Within the cellular component group, differentially expressed genes associated with the cell, cell part, membrane, and membrane part represented the largest clusters. In terms of molecular function, most differentially expressed genes were responsible for catalytic activity, binding, transporter activity, and structural molecule activity.

KEGG pathway analysis was also performed to reveal the interaction of different pathways during the process of ETR in *S.* Enteritidis. As presented in Fig. 3, differentially expressed genes were principally enriched in pathways of metabolism (e.g., carbohydrate metabolism, amino acid metabolism), environmental information processing (e.g., signal transduction, membrane transport), genetic information processing (e.g., transcription, translation), and cellular processes (e.g., cell motility). The top 20 enriched KEGG pathways were shown in Fig. 4 as a scatter plot. Moreover, a proposed model for the regulation of ETR in *S.* Enteritidis was illustrated in Fig. 5, of which the major metabolic pathways were discussed herein.

3.2.1. Metabolism

A considerable proportion of differentially expressed genes were associated with cellular metabolism in the current work (Fig. 3). These genes were mainly distributed in metabolic pathways for carbohydrates, amino acids, energy, cofactors, vitamins, nucleotide, xenobiotics, lipids, terpenoids, polyketides, and other secondary metabolites (Fig. 5). Such diverse gene expression alterations indicated that *S.* Enteritidis could coordinately regulate the metabolic processes of many macromolecules to adapt to sublethal ethanol stress.

It was noted that the expression of many genes related to carbohydrate metabolism showed differential expression in the current work. In particular, most fructose and mannose metabolic genes were up-regulated in response to sublethal ethanol stress (Table 2). Fructose and mannose have been suggested to confer resistance to lactic acid in *Escherichia coli* O157:H7 (Lan et al., 2022). These carbohydrates are required by foodborne pathogens as the main source of nutrients and energy, thus contributing to bacterial survival under stressful conditions.

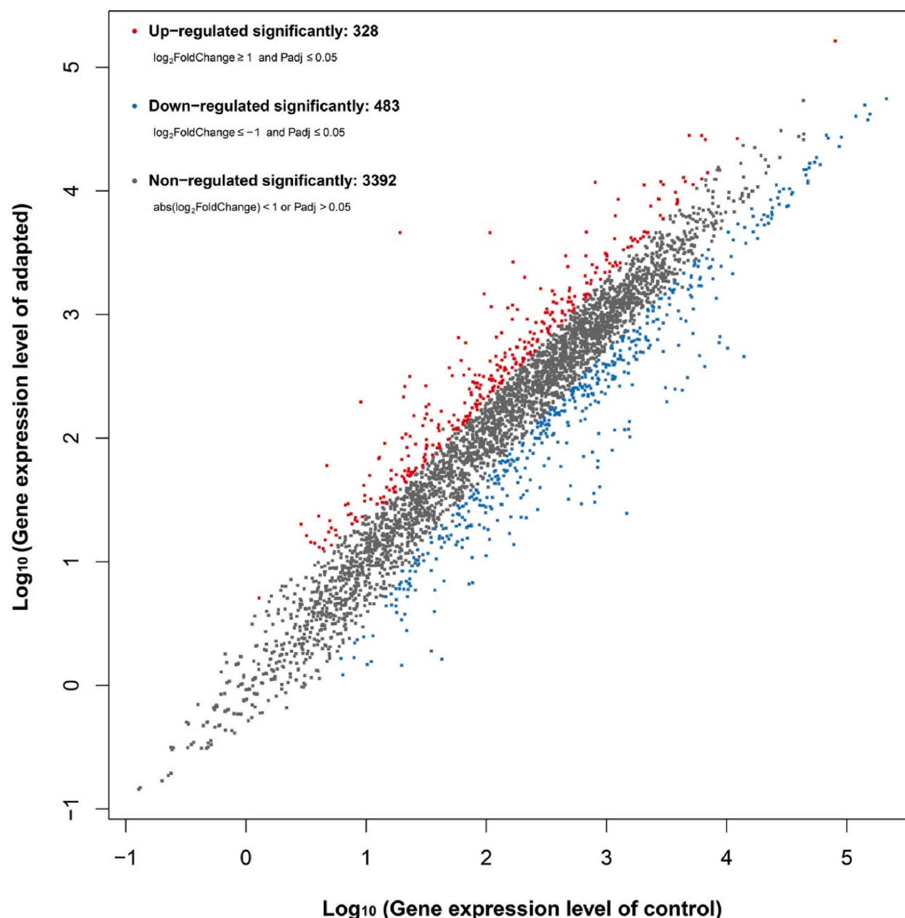

Fig. 1. Scatter plot of differentially expressed genes in *S.* Enteritidis under sublethal ethanol stress.

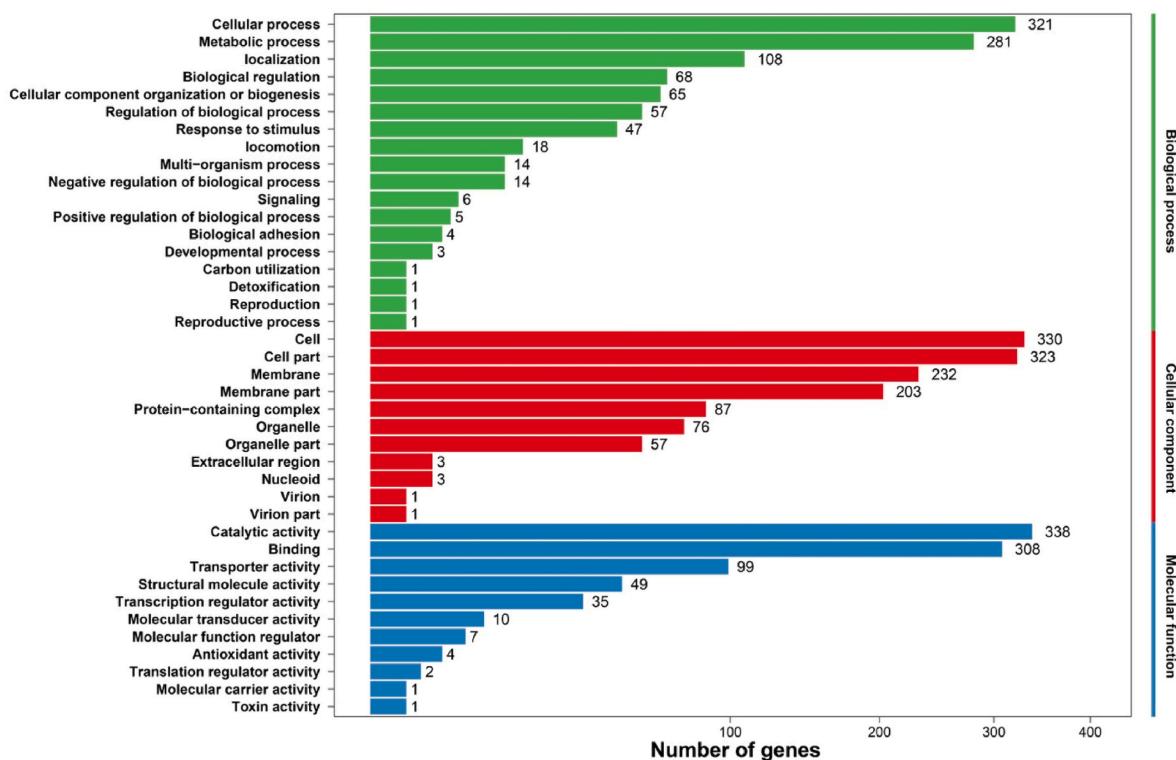

Fig. 2. GO functional analysis of differentially expressed genes in *S.* Enteritidis under sublethal ethanol stress.

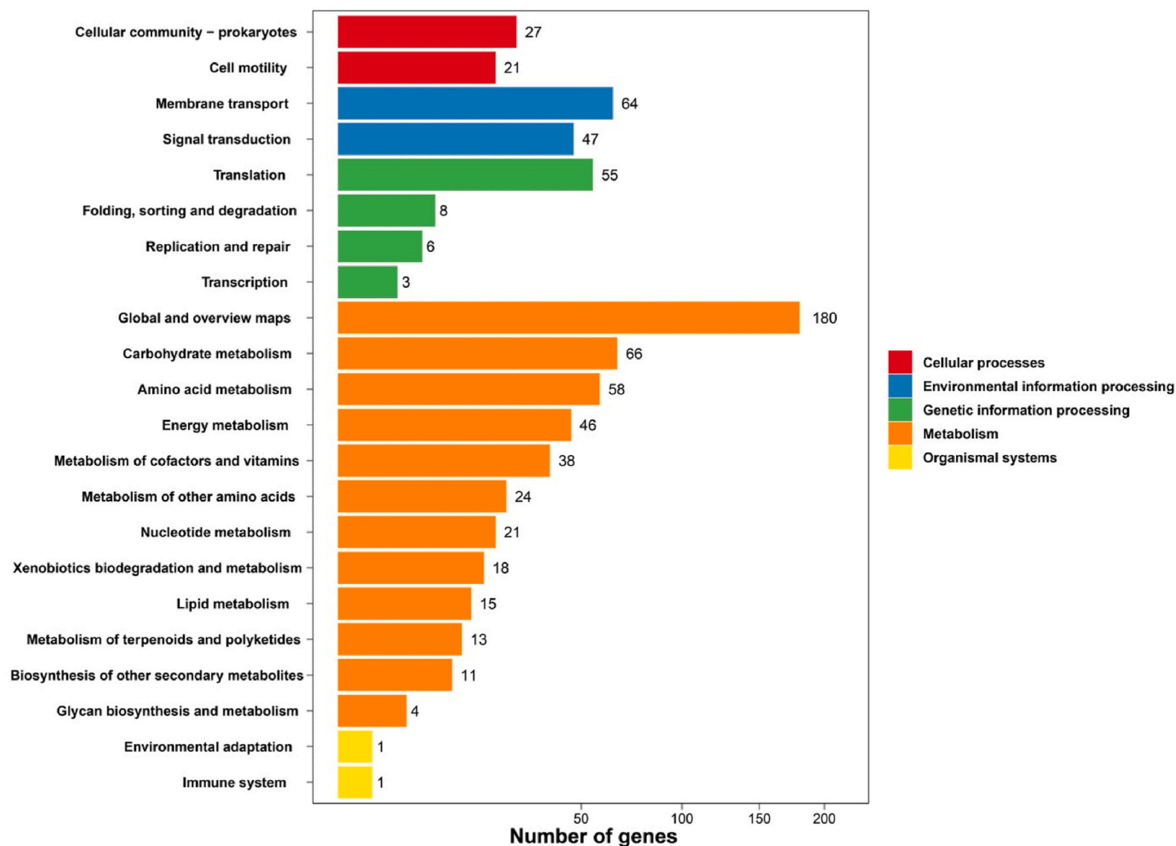

Fig. 3. KEGG pathway analysis of differentially expressed genes in *S.* Enteritidis under sublethal ethanol stress.

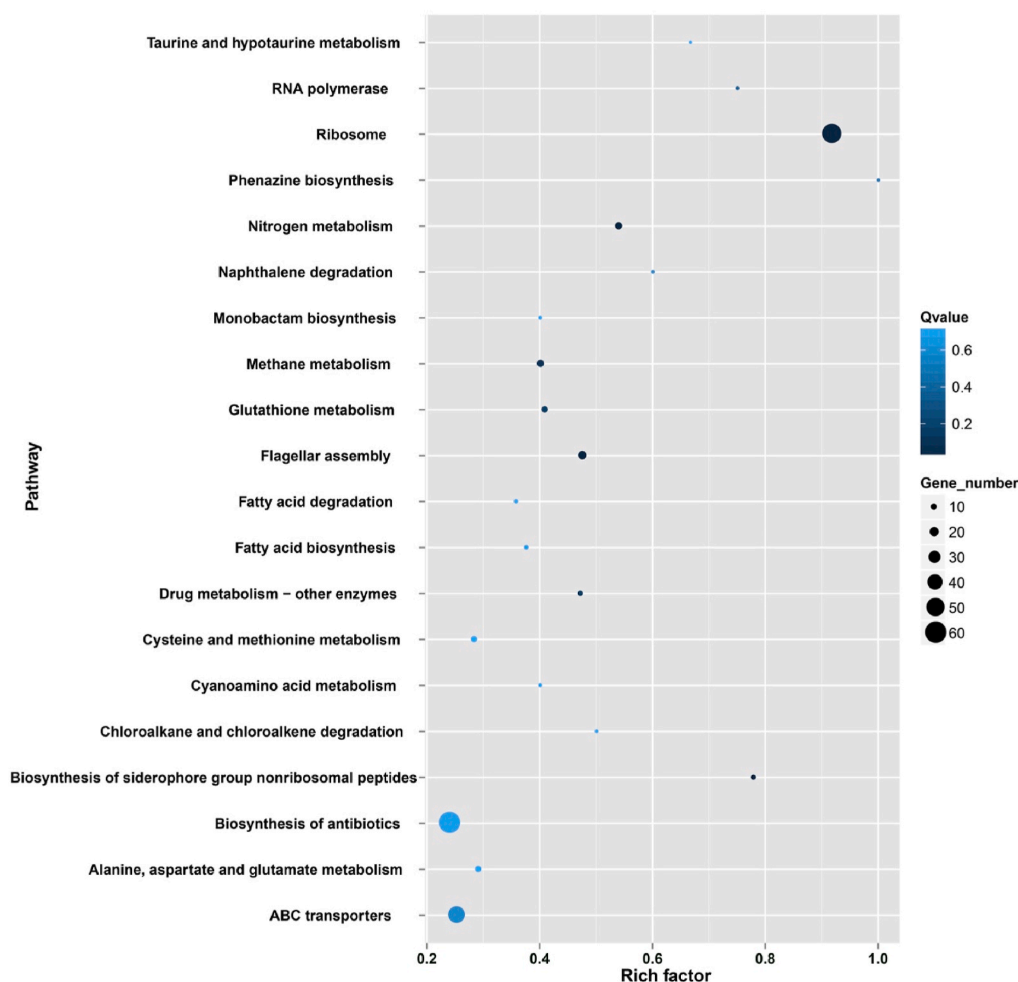

Fig. 4. Top 20 enriched KEGG pathways in *S.* Enteritidis under sublethal ethanol stress. Note: Plot size indicates the number of differentially expressed genes in a pathway. Plot color indicates Q_{value}, and means more obvious pathway enrichment as it becomes closer to bule. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2.2. Signal transduction

Bacterial two-component system is a crucial signal transduction pathway that consists of a histidine kinase that senses external stimulus and a cytoplasmic response regulator protein that modulates gene expression. In the current work, a total of 47 genes belonging to two-component systems showed altered expression in *S.* Enteritidis, and most of them were up-regulated in response to sublethal ethanol stress. Practically, the expression of several histidine kinase genes (e.g., *phoR*, *ssrA*, *baeS*, *glnL*, *ttrS*) and response regulator genes (e.g., *phoB*, *ssrB*) was enhanced by sublethal ethanol treatment (Table 2). These genes have been recognized as important sensing and signaling elements employed by *S. enterica* to survive under harsh conditions (de Pina et al., 2021). Interestingly, deletion of some two-component system genes (e.g., *phoP*, *degU*, *virS*, *yycG*, *agrC*, *liaS*) significantly impaired the growth of *L. monocytogenes* under ethanol stress (Pöntinen et al., 2017). Hence, it is reasonable to speculate that two-component systems play a role in the ETR of *S.* Enteritidis.

3.2.3. Membrane transport

In the membrane transport category, all three pathways (i.e., ABC transporters, phosphotransferase systems, bacterial secretion systems) had differentially expressed genes in response to sublethal ethanol treatment (Fig. 5). The largest number of differentially expressed genes was observed in the ABC transporter pathway, followed by the phosphotransferase system, and the bacterial secretion system. In terms of ABC transporters, up-regulated genes were mainly associated with transport of 2-aminoethylphosphonate (*phnS*, *phnV*, *phnU*, *phnT*), phosphate (*pstS*, *pstC*, *pstA*, *pstB*), osmoprotectant (*SEN1556*, *SEN1557*), oligopeptide (*oppA*, *oppB*, *oppC*, *oppD*), and glutamate/aspartate (*gltI*, *gltK*, *gltJ*), while down-regulated genes were mainly related to transport of iron complex (*fhuB*, *fhuC*, *fhuD*), manganese (*sitA*, *sitB*, *sitC*), and ribose (*rbsA*, *rbsD*) (Table 2). Such a sizable fraction of differentially expressed genes certainly highlights the importance of ABC transporters to the ETR in *S.* Enteritidis. Similarly, 2-aminoethylphosphonate transporter genes *phnSTUV* and phosphate transporter genes *pstSCAB* were overexpressed in *S.* Typhimurium during acid adaptation (Ryan et al., 2015). Therefore, it would be interesting to explore the role of ABC transporters in the ETR of *S.* Enteritidis in future studies.

Sublethal ethanol treatment also led to the differential expression of phosphotransferase system (PTS)-related genes in the current work (Table 2). In particular, mannose-PTS genes (e.g., *manX*, *manY*, *manZ*), fructose-PTS genes (e.g., *fruA*), and glucitol/sorbitol-PTS genes (e.g., *srlA*, *slrB*, *srlE*) went through an increased expression during the induction of ETR (Table 2). It was noted that the expression of many genes related to mannose and fructose metabolism was also activated by sublethal ethanol stress (Table 2). These observations suggested the importance of mannose- and fructose-PTS to the ETR in *S.* Enteritidis. Similarly, mannose-PTS genes *manXYZ* were involved in the response of *S. enterica* and *E. coli* to sodium hypochlorite and organic solvent, respectively (Okochi et al., 2007; Wang et al., 2010).

Several genes in the bacterial secretion pathway also showed altered expression, with *ssaJ*, *ssaN* and *SEN1635* in the type III secretion system

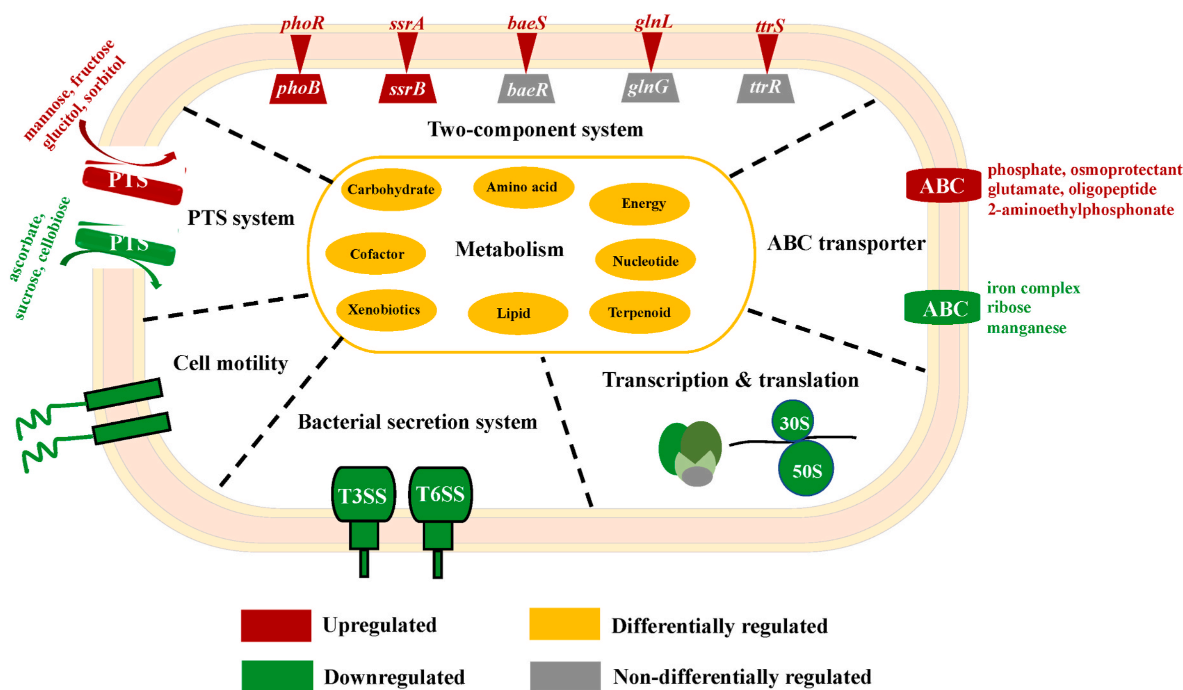

Fig. 5. Schematic diagram of main metabolic regulations in *S.* Enteritidis under sublethal ethanol stress.

(T3SS) up-regulated as well as *secE*, *secY*, and *yidC* in the type II secretion system, and *SEN1970* in the type VI secretion system down-regulated, respectively (Table 2). Similarly, the expression of many T3SS-related genes was also triggered in *S.* Enteritidis by acid adaptation (Hu et al., 2018). T3SS and other secretion systems are fundamental to the colonization and survival of *S. enterica* during infection in the animal host (Bao et al., 2020). It is thus indicative that *S.* Enteritidis may alter its virulence in stressful conditions such as ethanol exposure, which can be explored in future studies.

3.2.4. Transcription and translation

Modulation of gene transcription and translation is necessary for foodborne pathogens to respond to environmental changes. In the current work, many genes related to transcription and translation were significantly differentially expressed in *S.* Enteritidis in response to sublethal ethanol treatment (Fig. 5). In the transcription pathway, three RNA polymerase genes (i.e., *rpoA*, *rpoB*, *rpoC*) were down-regulated by 3.97-, 2.39- and 2.62-fold, respectively, indicating RNA transcribing function might be weakened under ethanol stress (Table 2). In terms of translation function, a total of 53 ribosome-related genes were repressed, including 33 large ribosomal subunit genes (e.g., *rplA*, *rpmA*) and 20 small ribosomal subunit genes (e.g., *rpsA*, *rpsB*) (Table 2). It thus seemed that the synthesis ability of ribosomes in *S.* Enteritidis was reduced in face of ethanol stress. In a similar vein, regulation of ribosome-related gene expression was also a strategy by which *E. coli* responds to ultrasonic stress and *C. sakazakii* copes with the combination of carvacrol and citral (Cao et al., 2020; Li et al., 2021).

3.2.5. Cell motility

In the case of cell motility function, the expression of genes responsible for bacterial chemotaxis and flagellar assembly was repressed by sublethal ethanol stress (Fig. 5). These bacterial chemotaxis genes mainly included aerotaxis receptor genes (e.g., *aer*), and methyl-accepting chemotaxis genes (e.g., *SEN3058*). Moreover, a total of 18 genes related to flagellar assembly were down-regulated in *S.* Enteritidis, such as flagellar basal body gene (e.g., *flgC*), flagellar hook gene (e.g., *flgE*), flagellar L-ring gene (e.g., *flgH*), flagellar M-ring gene (e.g., *fliF*), flagellar motor switch gene (e.g., *fliG*), and flagellar biosynthesis gene (e.g., *fliQ*) (Table 2). Similarly, flagellar assembly-related genes were also down-regulated in *S. enterica* in the presence of many other food processing-related stress factors such as chlorine, acid and heat (Ryan et al., 2015; Sirsat et al., 2011; Wang et al., 2010). It has been suggested that inhibition of flagellar assembly might be an energy conservation strategy that enabled bacterial survival under stressful conditions by reducing energy-consuming processes (Hu et al., 2018).

It should be noted that some of the aforementioned pathways such as metabolism, ABC transporters, and translation were also significantly differentially expressed in *S.* Enteritidis in response to sublethal ethanol treatment as revealed by proteomic analysis in our previous work (He et al., 2019). On the contrary, the involvement of two-component systems, PTS systems, and RNA polymerases in the ETR of *S.* Enteritidis was principally uncovered by transcriptomic analysis in the current work. Thus, transcriptomics may be a powerful tool for elucidating stress resistance mechanisms of pathogenic bacteria during food processing (Lamas et al., 2019).

3.3. Validation of differentially expressed genes by RT-qPCR

Several differentially expressed genes were subjected to RT-qPCR analysis in the current work. Gene expression profile in the RNA-seq and RT-qPCR tests was then compared. As shown in Fig. 6, the overall trend of the differential expression pattern for *asr*, *srlE*, *rbfA*, *SEN1805*, and *SEN1383* genes was similar as determined by both techniques. This finding provided evidence that the RNA-seq test was properly conducted and the resulting data were reliable.

4. Conclusion

Transcriptome sequencing revealed that multiple genes and adaptation pathways were involved in the ETR of *S.* Enteritidis. In total, 811 genes were significantly differentially expressed in response to sublethal ethanol treatment. A couple of two-component sensor and response genes were up-regulated to activate signaling pathways. The expression of ABC transporter genes responsible for transport of osmoprotectant, phosphate, and 2-aminoethylphosphonate were also induced as a membrane transport strategy. On the other hand, genes related to

Table 2
Selected differentially expressed genes mentioned in the Results and discussion section.

Gene ID	Gene name	Fold change	Description
Fructose and mannose metabolism			
SEN1205	*manZ*	6.52	PTS system mannose-specific transporter subunit IID
SEN1206	*manY*	5.13	Phosphotransferase enzyme II, C component
SEN2673	*srlA*	2.83	PTS system glucitol/sorbitol-specific transporter subunit IIBC
SEN2197	*fruA*	3.09	Fructose PTS system EIIA component
SEN2675	*slrB*	5.09	PTS system glucitol/sorbitol-specific transporter subunit IIA
SEN2674	*srlE*	4.91	PTS system glucitol/sorbitol-specific transporter subunit IIBC
SEN3875	*glpX*	−2.11	Fructose-1,6-bisphosphatase I
SEN2676	*srlD*	10.51	Sorbitol-6-phosphate 2-dehydrogenase
SEN1207	*manX*	2.61	PTS system mannose-specific transporter subunit IIAB
SEN2137	*fbaB*	2.60	Fructose-bisphosphate aldolase, class I
SEN1717	*pfkB*	3.33	6-phosphofructokinase 1
Two-component systems			
SEN0381	*phoR*	2.98	Phosphate regulon sensor protein
SEN0380	*phoB*	2.56	Transcriptional regulator PhoB
SEN1653	*ssrA*	2.76	Two-component sensor kinase
SEN1654	*ssrB*	3.07	Two-component response regulator
SEN2126	*baeS*	3.07	Signal transduction histidine-protein kinase
SEN3794	*glnL*	2.04	Nitrogen regulation protein NR (II)
SEN1659	*ttrS*	2.01	Histidine kinase, two component regulatory protein
ABC transporters			
SEN0411	*phnS*	4.17	Periplasmic binding component of 2-amino-ethylphosphonate transporter
SEN0408	*phnV*	2.38	Membrane protein of 2-aminoethylphosph-onate transporter
SEN0409	*phnU*	3.66	Membrane protein of 2-aminoethylphosph-onate transporter
SEN0410	*phnT*	2.21	2-aminoethylphosphonate transporter ATP-binding protein
SEN3671	*pstS*	15.88	Phosphate ABC transporter substrate-binding protein
SEN3670	*pstC*	7.48	Phosphate transporter permease subunit PstC
SEN3669	*pstA*	7.20	Phosphate transporter permease subunit PtsA
SEN3668	*pstB*	3.92	Phosphate transporter ATP-binding protein
SEN1556	*SEN1556*	2.93	ABC transporter membrane protein
SEN1557	*SEN1557*	2.32	ABC transporter substrate-binding protein
SEN1289	*oppA*	2.16	Periplasmic oligopeptide-binding protein OppA
SEN1290	*oppB*	2.26	Oligopeptide transporter permease
SEN1291	*oppC*	2.10	Oligopeptide transport system permease OppC
SEN1292	*oppD*	2.08	Oligopeptide transporter ATP-binding protein
SEN0634	*gltI*	3.30	Glutamate and aspartate transporter subunit
SEN0632	*gltK*	2.44	Glutamate/aspartate transport system permease GltK
SEN0633	*gltJ*	2.31	Glutamate/aspartate transport system permease GltJ
SEN0199	*fhuB*	−3.21	Iron-hydroxamate transporter permease subunit
SEN0197	*fhuC*	−2.55	Iron-hydroxamate transporter ATP-binding subunit
SEN0198	*fhuD*	−2.88	Iron-hydroxamate transporter substrate-binding subunit
SEN2703	*sitA*	−3.32	Iron transport protein periplasmic-binding protein
SEN2704	*sitB*	−2.39	Iron transport protein ATP-binding protein
SEN2705	*sitC*	−2.22	Iron transport protein inner membrane protein
SEN3696	*rbsA*	−9.10	D-ribose transporter ATP-binding protein
SEN3695	*rbsD*	−15.11	D-ribose pyranase
Phosphotransferase systems			
SEN1207	*manX*	2.16	PTS system mannose-specific transporter subunit IIAB
SEN1206	*manY*	5.13	Phosphotransferase enzyme II, C component
SEN1205	*manZ*	6.52	PTS system mannose-specific transporter subunit IID
SEN2197	*fruA*	3.09	Fructose PTS system EIIA component
SEN2673	*srlA*	2.83	PTS system glucitol/sorbitol-specific transporter subunit IIBC
SEN2675	*slrB*	5.09	PTS system glucitol/sorbitol-specific transporter subunit IIA
SEN2674	*srlE*	4.91	PTS system glucitol/sorbitol-specific transporter subunit IIBC
Bacterial secretion systems			
SEN1636	*ssaJ*	2.29	Pathogenicity island lipoprotein
SEN1630	*ssaN*	2.22	Type III secretion system ATPase
SEN1635	*SEN1635*	2.80	Pathogenicity island protein
SEN3931	*secE*	−2.11	Preprotein translocase subunit SecE
SEN3248	*secY*	−3.85	Preprotein translocase subunit SecY
SEN3659	*yidC*	−3.20	Inner membrane protein translocase component YidC
SEN1970	*SEN1970*	−4.56	Phage integrase
Transcription			
SEN3243	*rpoA*	−3.97	DNA-directed RNA polymerase subunit alpha
SEN3937	*rpoB*	−2.39	DNA-directed RNA polymerase subunit beta
SEN3938	*rpoC*	−2.62	DNA-directed RNA polymerase subunit beta'
Translation			
SEN3254	*rpsH*	−4.10	30 S ribosomal protein S8
SEN3247	*rpmJ*	−4.49	50 S ribosomal protein L36
SEN3178	*rplM*	−3.19	50 S ribosomal protein L13
SEN3258	*rplN*	−2.48	50 S ribosomal protein L14
SEN2597	*rpsP*	−3.06	30 S ribosomal protein S16
SEN3934	*rplA*	−3.52	50 S ribosomal protein L1
SEN3275	*rpsG*	−3.56	30 S ribosomal protein S7
SEN3260	*rpmC*	−3.70	50 S ribosomal protein L29
SEN3253	*rplF*	−3.02	50 S ribosomal protein L6
SEN3886	*rpmE*	−3.41	50 S ribosomal protein L31
SEN3266	*rplW*	−3.64	50 S ribosomal protein L23
SEN3276	*rpsL*	−3.01	30 S ribosomal protein S12
SEN3118	*rpsO*	−4.51	30 S ribosomal protein S15
SEN4160	*rplL*	−3.54	50 S ribosomal protein L9
SEN3257	*rplX*	−3.09	50 S ribosomal protein L24
SEN3136	*rpmA*	−3.44	50 S ribosomal protein L27
SEN4157	*rpsF*	−3.30	30 S ribosomal protein S6
SEN3549	*rpmG*	−2.16	50 S ribosomal protein L33
SEN3246	*rpsM*	−3.41	30 S ribosomal protein S13
SEN3259	*rpsQ*	−4.77	30 S ribosomal protein S17
SEN0450	*rpmE2*	−5.27	50 S ribosomal protein L31
SEN3137	*rplU*	−2.89	50 S ribosomal protein L21
SEN3269	*rpsJ*	−3.67	30 S ribosomal subunit protein S10
SEN3245	*rpsK*	−3.34	30 S ribosomal protein S11
SEN3177	*rpsL*	−2.83	30 S ribosomal protein S9
SEN4159	*rpsR*	−3.70	30 S ribosomal protein S18
SEN3244	*rpsD*	−3.96	30 S ribosomal protein S4
SEN3264	*rpsS*	−4.08	30 S ribosomal protein S19
SEN3267	*rplD*	−3.17	50 S ribosomal protein L4
SEN3550	*rpmB*	−2.40	50 S ribosomal protein L28
SEN3263	*rplV*	−4.37	50 S ribosomal protein L22
SEN0043	*rpsT*	−3.49	30 S ribosomal protein S20
SEN3935	*rplJ*	−3.32	50 S ribosomal protein L10
SEN2594	*rplS*	−3.71	50 S ribosomal protein L19
SEN3250	*rpmD*	−4.40	50 S ribosomal protein L30
SEN1708	*rplT*	−2.80	50 S ribosomal protein L20
SEN0223	*rpsB*	−2.97	30 S ribosomal protein S2
SEN3242	*rplQ*	−4.30	50 S ribosomal protein L17
SEN3262	*rpsC*	−3.69	30 S ribosomal protein S3
SEN3656	*rpmH*	−3.17	50 S ribosomal protein L34
SEN3265	*rplB*	−3.80	50 S ribosomal protein L2
SEN3256	*rplE*	−3.10	50 S ribosomal protein L5
SEN3936	*rplL*	−3.73	50 S ribosomal protein L7/L12
SEN1709	*rpmI*	−2.99	50 S ribosomal protein L35
SEN0885	*rpsA*	−2.85	30 S ribosomal protein S1
SEN3252	*rplR*	−3.90	50 S ribosomal protein L18
SEN3261	*rplP*	−3.83	50 S ribosomal protein L16
SEN3249	*rplO*	−3.40	50 S ribosomal protein L15
SEN3255	*rpsN*	−3.96	30 S ribosomal protein S14
SEN3933	*rplK*	−2.87	50 S ribosomal protein L11

(continued on next page)

Table 2 (*continued*)

Gene ID	Gene name	Fold change	Description
SEN3268	*rplC*	−2.95	50 S ribosomal protein L3
SEN2217	*rplY*	−2.95	50 S ribosomal protein L25
SEN3251	*rpsE*	−3.72	30 S ribosomal protein S5
Cell motility			
SEN3059	*aer*	−2.01	Aerotaxis receptor protein
SEN3058	*SEN3058*	−2.68	Methyl-accepting chemotaxis protein II
SEN1033	*fliM*	−2.86	Flagellar motor switch protein FliM
SEN1032	*fliN*	−2.11	Flagellar motor switch protein FliN
SEN1039	*fliG*	−2.62	Flagellar motor switch protein G
SEN1031	*fliO*	−2.17	Flagellar biosynthesis protein FliO
SEN1868	*flgH*	−2.20	Flagellar basal body L-ring protein
SEN1871	*flgE*	−2.05	Flagellar hook protein FlgE
SEN1029	*fliQ*	−2.40	Flagellar biosynthesis protein FliQ
SEN1028	*fliR*	−5.74	Flagellar biosynthesis protein FliR
SEN1875	*flgA*	−2.55	Flagellar basal body P-ring biosynthesis protein FlgA
SEN1872	*flgD*	−2.10	Flagellar basal body rod modification protein
SEN1040	*fliF*	−2.50	Flagellar MS-ring protein
SEN1873	*flgC*	−2.30	Flagellar basal body rod protein FlgC
SEN1036	*fliJ*	−3.32	Flagellar biosynthesis chaperone
SEN1037	*fliL*	−2.48	Flagellum-specific ATP synthase
SEN1869	*flgG*	−2.21	Flagellar basal body rod protein FlgG
SEN1038	*fliH*	−2.22	Flagellar assembly protein H
SEN1030	*fliP*	−2.39	Flagellar biosynthesis protein FliP
SEN1870	*flgF*	−2.20	Flagellar basal body rod protein FlgF

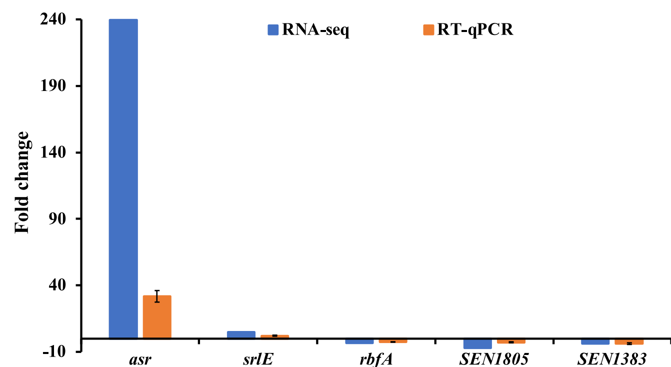

Fig. 6. Comparison of gene expression patterns revealed by the RNA-seq and RT-qPCR tests.

ribosomal units, bacterial chemotaxis, and flagellar assembly were repressed to serve as a possible energy conservation strategy. These findings offer new insight into the ETR mechanisms in *S.* Enteritidis at the transcriptome level. Future work can be focused on the functional characterization of differentially expressed genes to further identify key regulatory networks governing bacterial ETR.

CRediT authorship contribution statement

Shoukui He: Writing – original draft, Methodology, Investigation, Funding acquisition. **Yan Cui:** Formal analysis, Writing – review & editing. **Rui Dong:** Methodology, Validation. **Jiang Chang:** Data curation, Visualization. **Hua Cai:** Data curation. **Hong Liu:** Writing – review & editing. **Xianming Shi:** Conceptualization, Supervision, Writing – review & editing, 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.

Acknowledgement

This work was supported by the National Key Research and Development Program of China (No. 2019YFE0119700), the National Natural Science Foundation of China (No. 32001797), the Natural Science Foundation of Shanghai (No. 22ZR1429900), and Shanghai Three-year Action Plan for Strengthening Public Health System Construction (2020–2022)-Capacity Building of Public Health on Comprehensive Monitoring, Prewarning System and Emergency Response in Megacity (GWV 1.1).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.04.011.

References

Bao, H., Wang, S., Zhao, J.H., Liu, S.L., 2020. *Salmonella* secretion systems: differential roles in pathogen-host interactions. Microbiol. Res. 241, 126591. https://doi.org/10.1016/j.micres.2020.126591.

Browne, N., Dowds, B.C.A., 2001. Heat and salt stress in the food pathogen *Bacillus cereus*. J. Appl. Microbiol. 91 (6), 1085–1094. https://doi.org/10.1046/j.1365-2672.2001.01478.x.

Browne, N., Dowds, B.C.A., 2002. Acid stress in the food pathogen *Bacillus cereus*. J. Appl. Microbiol. 92 (3), 404–414. https://doi.org/10.1046/j.1365-2672.2002.01541.x.

Cao, Y., Zhou, A., Zhou, D., Xiao, X., Yu, Y., Li, X., 2020. *Cronobacter sakazakii* CICC 21544 responds to the combination of carvacrol and citral by regulating proton motive force. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 122, 109040. https://doi.org/10.1016/j.lwt.2020.109040.

Chiang, M.L., Ho, W.L., Chou, C.C., 2006. Response of *Vibrio parahaemolyticus* to ethanol shock. Food Microbiol. 23 (5), 461–467. https://doi.org/10.1016/j.fm.2005.07.001.

Chiang, M.L., Ho, W.L., Chou, C.C., 2008. Ethanol shock changes the fatty acid profile and survival behavior of *Vibrio parahaemolyticus* in various stress conditions. Food Microbiol. 25 (2), 359–365. https://doi.org/10.1016/j.fm.2007.10.002.

Crucello, A., Furtado, M.M., Chaves, M.D., Sant'Ana, A.S., 2019. Transcriptome sequencing reveals genes and adaptation pathways in *Salmonella* Typhimurium inoculated in four low water activity foods. Food Microbiol. 82, 426–435. https://doi.org/10.1016/j.fm.2019.03.016.

Dao, T., Dantigny, P., 2011. Control of food spoilage fungi by ethanol. Food Control 22 (3–4), 360–368. https://doi.org/10.1016/j.foodcont.2010.09.019.

de Pina, L.C., da Silva, F.S.H., Galvão, T.C., Pauer, H., Ferreira, R.B.R., Antunes, L.C.M., 2021. The role of two-component regulatory systems in environmental sensing and virulence in *Salmonella*. Crit. Rev. Microbiol. 47 (4), 397–434. https://doi.org/10.1080/1040841X.2021.1895067.

Deng, X., Li, Z., Zhang, W., 2012. Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. Food Microbiol. 30 (1), 311–315. https://doi.org/10.1016/j.fm.2011.11.001.

Dev Kumar, G., Mishra, A., Dunn, L., Townsend, A., Oguadinma, I.C., Bright, K.R., Gerba, C.P., 2020. Biocides and novel antimicrobial agents for the mitigation of coronaviruses. Front. Microbiol. 11, 1351. https://doi.org/10.3389/fmicb.2020.01351.

Doulia, D., Katsinis, G., Mougin, B., 2000. Prolongation of the microbial shelf life of wrapped part baked baguettes. Int. J. Food Prop. 3 (3), 447–457. https://doi.org/10.1080/10942910009524648.

Fagerlund, A., Møretrø, T., Heir, E., Briandet, R., Langsrud, S., 2017. Cleaning and disinfection of biofilms composed of *Listeria monocytogenes* and background microbiota from meat processing surfaces. Appl. Environ. Microbiol. 83 (17), e01046–17. https://doi.org/10.1128/AEM.01046-17.

He, S., Fong, K., Wang, S., Shi, X., 2021a. Ethanol adaptation in foodborne bacterial pathogens. Crit. Rev. Food Sci. Nutr. 61 (5), 777–787. https://doi.org/10.1080/10408398.2020.1746628.

He, S., Fong, K., Wang, S., Shi, X., 2021b. Meat juice contributes to the stability of ethanol adaptation in *Salmonella enterica* serovar Enteritidis. Food Qual. Saf. 5, 1–6. https://doi.org/10.1093/fqsafe/fyab017.

He, S., Qin, X., Wong, C.W., Shi, C., Wang, S., Shi, X., 2019. Ethanol adaptation strategies in *Salmonella enterica* serovar Enteritidis revealed by global proteomic and mutagenic analyses. Appl. Environ. Microbiol. 85 (19), e01107–e01119. https://doi.org/10.1128/AEM.01107-19.

He, S., Zhou, X., Shi, C., Shi, X., 2016. Ethanol adaptation induces direct protection and cross-protection against freezing stress in *Salmonella enterica* serovar Enteritidis. J. Appl. Microbiol. 120 (3), 697–704. https://doi.org/10.1111/jam.13042.

Hingston, P., Chen, J., Allen, K., Truelstrup Hansen, L., Wang, S., 2017. Strand specific RNA-sequencing and membrane lipid profiling reveals growth phase-dependent cold stress response mechanisms in *Listeria monocytogenes*. PLoS One 12 (6), e0180123. https://doi.org/10.1371/journal.pone.0180123.

Hu, S., Yu, Y., Zhou, D., Li, R., Xiao, X., Wu, H., 2018. Global transcriptomic acid tolerance response in *Salmonella* Enteritidis. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 92, 330–338. https://doi.org/10.1016/j.lwt.2018.02.039.

Huang, X., Zhou, X., Jia, B., Li, N., Jia, J., He, M., Shi, X., 2019. Transcriptional sequencing uncovers survival mechanisms of *Salmonella enterica* serovar Enteritidis

in antibacterial egg white. mSphere 4 (1), e00700–e00718. https://doi.org/10.1128/mSphere.00700-18.

Huang, Y.T., Cheng, K.C., Yu, R.C., Chou, C.C., 2013. Effect of ethanol shock pretreatment on the tolerance of *Cronobacter sakazakii* BCRC 13988 exposed to subsequent lethal stresses. Foodb. Pathog. Dis. 10 (2), 165–170. https://doi.org/10.1089/fpd.2012.1291.

Katsinis, G., Rigas, F., Doulia, D., 2008. Synergistic effect of chemical preservatives with ethanol on the microbial shelf life of bread by factorial design. Int. J. Food Sci. Technol. 43, 208–215. https://doi.org/10.1111/j.1365-2621.2006.01386.x.

Lamas, A., Regal, P., Vázquez, B., Miranda, J.M., Franco, C.M., Cepeda, A., 2019. Transcriptomics: a powerful tool to evaluate the behavior of foodborne pathogens in the food production chain. Food Res. Int. 125, 108543. https://doi.org/10.1016/j.foodres.2019.108543.

Lan, L., Shi, H., 2022. New insights into the formation and recovery of sublethally injured *Escherichia coli* O157:H7 induced by lactic acid. Food Microbiol. 102, 103918. https://doi.org/10.1016/j.fm.2021.103918.

Li, J., Liu, D., Ding, T., 2021. Transcriptomic analysis reveal differential gene expressions of *Escherichia coli* O157: H7 under ultrasonic stress. Ultrason. Sonochem. 71, 105418. https://doi.org/10.1016/j.ultsonch.2020.105418.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$method. Methods 25 (4), 402–408. https://doi.org/10.1006/meth.2001.1262.

Lou, Y., Yousef, A.E., 1997. Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. Appl. Environ. Microbiol. 63 (4), 1252–1255. https://doi.org/10.1128/aem.63.4.1252-1255.1997.

Okochi, M., Kurimoto, M., Shimizu, K., Honda, H., 2007. Increase of organic solvent tolerance by overexpression of *manXYZ* in *Escherichia coli*. Appl. Microbiol. Biotechnol. 73, 1394–1399. https://doi.org/10.1007/s00253-006-0624-y.

Park, J.Y., Jo, S.K., Park, K.M., Yu, H., Bai, J., Ryu, S., Chang, P.S., 2019. Transcriptomic analysis of *Staphylococcus aureus* under the stress condition of antibacterial erythorbyl laurate by RNA sequencing. Food Control 96, 1–8. https://doi.org/10.1016/j.foodcont.2018.08.021.

Pöntinen, A., Lindström, M., Skurnik, M., Korkeala, H., 2017. Screening of the two-component-system histidine kinases of *Listeria monocytogenes* EGD-e. LiaS is needed for growth under heat, acid, alkali, osmotic, ethanol and oxidative stresses. Food Microbiol. 65, 36–43. https://doi.org/10.1016/j.fm.2017.01.018.

Ryan, D., Pati, N.B., Ojha, U.K., Padhi, C., Ray, S., Jaiswal, S., Suar, M., 2015. Global transcriptome and mutagenic analyses of the acid tolerance response of *Salmonella enterica* serovar Typhimurium. Appl. Environ. Microbiol. 81 (23), 8054–8065. https://doi.org/10.1128/AEM.02172-15.

Shen, J., Duan, H.Y., Wang, J.Q., Li, T., Yao, X.Y., 2016. Investigation on cleaning and disinfection status of food enterprises in China. Chin. J. Disinfection 33 (10), 1009–1011. https://doi.org/10.11726/j.issn.1001-7658.2016.10.026 (in Chinese).

Shibasaki, I., 1982. Food preservation with nontraditional antimicrobial agents. J. Food Saf. 4, 35–58. https://doi.org/10.1111/j.1745-4565.1982.tb00432.x.

Sirsat, S.A., Burkholder, K.M., Muthaiyan, A., Dowd, S.E., Bhunia, A.K., Ricke, S.C., 2011. Effect of sublethal heat stress on *Salmonella* Typhimurium virulence. J. Appl. Microbiol. 110 (3), 813–822. https://doi.org/10.1111/j.1365-2672.2011.04941.x.

Wang, S., Phillippy, A.M., Deng, K., Rui, X., Li, Z., Tortorello, M.L., Zhang, W., 2010. Transcriptomic responses of *Salmonella enterica* serovars Enteritidis and Typhimurium to chlorine-based oxidative stress. Appl. Environ. Microbiol. 76 (15), 5013–5024. https://doi.org/10.1128/AEM.00823-10.

Weerasooriya, G., McWhorter, A.R., Khan, S., Chousalkar, K.K., 2021. Transcriptomic response of *Campylobacter jejuni* following exposure to acidified sodium chlorite. npj Sci. Food 5, 1–9. https://doi.org/10.1038/s41538-021-00103-5.

Yeh, Y., 2012. Effect of Sub-lethal Ethanol Treatment on the Protein Expression of *Cronobacter Sakazakii* and its Viability during Exposure to Subsequent Lethal Stresses [Master Thesis]. National Taiwan University, Taipei.

Zhou, A., Cao, Y., Zhou, D., Hu, S., Tan, W., Xiao, X., Li, X., 2020. Global transcriptomic analysis of *Cronobacter sakazakii* CICC 21544 by RNA-seq under inorganic acid and organic acid stresses. Food Res. Int. 130, 108963. https://doi.org/10.1016/j.foodres.2019.108963.