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

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**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 adaptive 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...
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, erythorbylurate, 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 centrifuged 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 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^ΔΔCt method with 16 S rRNA as the reference gene (Livak and Schmittgen, 2001).

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 0-1000 -s 28-32 -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* strain. Relative expression level of each gene in ethanol-treated samples compared with control samples was calculated by 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/). The resulting data were then utilized 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^ΔΔCt 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’) |
|-----------------------------------|------|---------------------------------|-------------------------------|
| *ar*                              | CGGTTGAGTATGGTGGTTT | CAGGCGCCGAGCCACTTCCG |
| *sFE*                             | ATCCCGAGCAGGGG   | GGGCGAGCATGTTCTGGAAAG |
| *rFl*                             | AAAAGCGGGGATCAA | AGCGCATCCCTTCCAGCC |
| *SEN1805*                         | TGATTGTGAGGGGTGGA | GGCGTCTGGTTCTGCTGTTT |
| *SEN1383*                         | GGCGTTGAGGATGGTGGTTA | AGGGACGATGTTCTGGAAAG |
| 16S rRNA                          | CAGAAGAAGCACCAGCTAAC | GACTAAGCCGACAGTCTTT |
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 $\geq 2$ 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.
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 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-aminoethoxyphosphonate (*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 (*rhaA*, *rhaD*) (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., *srIA*, *srIB*, *srIIE*) 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.
(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 cope 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., flgL), flagellar M-ring gene (e.g., flIP), 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, srkE, 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 |
|---------|-----------|-------------|-------------|
| SEN1190 | manY      | 5.13        | Phosphotransferase enzyme II, C component |
| SEN1205 | manZ      | 6.52        | PTS system mannos-specific transporter subunit IID |
| SEN2197 | fhuA      | 3.09        | Fructose PTS system EI component |
| SEN2673 | srA       | 2.83        | PTS system glucitol/sorbitol-specific transporter subunit IBIC |
| SEN2675 | srB       | 5.09        | PTS system glucitol/sorbitol-specific transporter subunit IIA |
| SEN2674 | srE       | 4.91        | PTS system glucitol/sorbitol-specific transporter subunit IIB |
| SEN3875 | gpx       | -2.11       | Fructose-1,6-biphosphatase 1 |
| SEN2676 | sitD      | 10.51       | Sorbitol-6-phosphate 2-dehydrogenase |
| SEN1207 | manX      | 2.61        | PTS system mannos-specific transporter subunit IIAB |
| SEN2137 | fbaB      | 2.60        | Fructose-biphosphatase aldolase, class I |
| SEN1717 | pflB      | 3.33        | 6-phosphofructokinase 1 |

Two-component systems

SEN0381 | pbpA | 2.98 | Phosphate regulon sensor protein |
SEN0380 | pbpB | 2.56 | Transcriptional regulator PbpB |
SEN1653 | sosA | 2.76 | Two-component sensor kinase |
SEN1654 | sosB | 3.07 | Two-component response regulator |
SEN2126 | batS | 3.07 | Signal transduction histidine-protein kinase |
SEN3794 | gbdL | 2.04 | Nitrogen regulation protein NR (II) |
SEN1659 | trsS | 2.01 | Histidine kinase, two component regulatory protein |

ABC transporters

SEN0411 | pbmS | 4.17 | Periplasmic binding component of 2-amino-ethylphosphonate transporter |
SEN0408 | phnU | 2.38 | Membrane protein of 2-aminoethylphosphonate transporter |
SEN0409 | phnT | 3.66 | Membrane protein of 2-aminoethylphosphonate transporter |

SEN0410 | phnT | 2.21 | 2-aminoethylphosphonate transporter ATP-binding protein |
SEN3671 | psaS | 15.88 | Phosphate ABC transporter substrate-binding protein |
SEN3670 | pscC | 7.48 | Phosphate transporter permease subunit PscC |
SEN3669 | ptsA | 7.20 | Phosphate transporter permease subunit PtsA |
SEN3668 | ptsB | 3.92 | Phosphate transporter ATP-binding protein |
SEN1556 | SEN1556 | 2.93 | ABC transporter membrane protein |
SEN1557 | SEN1557 | 2.22 | 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 transporter permease subunit OppC |
SEN1292 | oppD | 2.08 | Oligopeptide transporter ATP-binding protein |

SEN0634 | gdi | 3.30 | Glutamate and aspartate transporter subunit |
SEN0632 | gdr | 2.44 | Glutamate/aspartate transport system permease Gdr |
SEN0633 | glj | 2.31 | Glutamate/aspartate transport system permease Glj |

SEN0199 | fhuA | -3.21 | Iron-hydroxamate transporter subunit |
SEN197 | fhuC | -2.55 | Iron-hydroxamate transporter ATP-binding subunit |
SEN198 | 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 | rbaA | -9.10 | D-ribose transporter ATP-binding protein |
SEN3695 | rboD | -15.11 | D-ribose pyranose transport system |

Phosphotransferase systems

SEN1207 | manX | 2.16 | PTS system mannos-specific transporter subunit IIAB |

Table 2 (continued)

| Gene ID | Gene name | Fold change | Description |
|---------|-----------|-------------|-------------|
| SEN3875 | gpx       | -2.11       | Fructose-1,6-biphosphatase 1 |
| SEN0199 | fhuA      | -3.21       | Iron-hydroxamate transporter subunit |
| SEN197  | fhuC      | -2.55       | Iron-hydroxamate transporter ATP-binding subunit |
| SEN198  | 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 | rbaA      | -9.10       | D-ribose transporter ATP-binding protein |
| SEN3695 | rboD      | -15.11      | D-ribose pyranose transport system |

Phosphotransferase systems

SEN1207 | manX | 2.16 | PTS system mannos-specific transporter subunit IIAB |
Table 2 (continued)

| Gene ID | Gene name | Fold change | Description |
|---------|-----------|-------------|-------------|
| SEN3268 | rplC      | -2.95       | 50 S ribosomal protein L3 |
| SEN2217 | rplV      | -2.95       | 50 S ribosomal protein L25 |
| SEN3251 | rpsE      | -3.72       | 30 S ribosomal protein S5 |
| SEN3059 | arr       | -2.01       | Aerotaxis receptor protein |
| SEN3058 | SEN3058   | -2.68       | Methyl-accepting chemotaxis protein II |
| SEN1033 | fIM       | -2.86       | Flagellar motor switch protein FIM |
| SEN1032 | fIN       | -2.11       | Flagellar motor switch protein FdN |
| SEN1039 | fIG       | -2.62       | Flagellar motor switch protein G |
| SEN1031 | fIO       | -2.17       | Flagellar biosynthesis protein FlgO |
| SEN1868 | flgH      | -2.20       | Flagellar basal body L-ring protein |
| SEN1871 | flgE      | -2.05       | Flagellar hook protein FlgE |
| SEN1029 | flgQ      | -2.40       | Flagellar biosynthesis protein FlgQ |
| SEN1028 | flgR      | -5.74       | Flagellar biosynthesis protein FlgR |
| SEN1875 | flgA      | -2.55       | Flagellar basal body P-ring biosynthesis protein FlgA |
| SEN1872 | flgD      | -2.10       | Flagellar basal body rod modification protein |
| SEN1040 | flfI      | -2.50       | Flagellar MS-ring protein |
| SEN1873 | flgC      | -2.30       | Flagellar basal body rod protein FlgC |
| SEN1036 | fIl       | -3.32       | Flagellar biosynthesis chaperone |
| SEN1037 | fIl       | -2.48       | Flagellum-specific ATP synthase |
| SEN1869 | flgF      | -2.27       | Flagellar basal body rod protein FlgF |
| SEN1038 | fIlH      | -2.03       | Flagellar assembly protein H |
| SEN1030 | fIlP      | -2.39       | Flagellar biosynthesis protein FlgP |
| SEN1870 | flgO      | -2.20       | Flagellar basal body rod protein FlgO |

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

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Appendix A. Supplementary data

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

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