Comprehensive evaluation and analysis of the salinity stress response mechanisms based on transcriptome and metabolome of *Staphylococcus aureus*

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Abstract

*Staphylococcus aureus* possesses an extraordinary ability to deal with a wide range of osmotic pressure. This study performed transcriptomic and metabolomic analyses on the potential mechanism of gradient salinity stress adaptation in *S. aureus* ZS01. The results revealed that CPS biosynthetic protein genes were candidate target genes for directly regulating the phenotypic changes of biofilm. Inositol phosphate metabolism was downregulated to reduce the conversion of functional molecules. The gluconeogenesis pathway and histidine synthesis were downregulated to reduce the production of endogenous glucose. The pyruvate metabolism pathway was upregulated to promote the accumulation of succinate. TCA cycle metabolism pathway was downregulated to reduce unnecessary energy loss. L-Proline was accumulated to regulate osmotic pressure. Therefore, these self-protection mechanisms can protect cells from hypertonic environments and help them focus on survival. In addition, we identified ten hub genes. The findings will aid in the prevention and treatment strategies of *S. aureus* infections.

Keywords *Staphylococcus aureus* · Salinity stress · Transcriptome · Metabolome

Introduction

*Staphylococcus aureus* is an important opportunistic pathogen, which is highly resistant to osmotic stress (Schuster et al. 2016). *S. aureus* strains can generate a large scale of toxic extracellular proteins while growing or occurring in food, resulting in the outbreaks of staphylococcal food poisoning (SFP) in humans and animals (Alibayov et al. 2014). SFP is a worldwide public health problem, mainly caused by enterotoxin, hemolysin, and leukotoxin (Loir et al. 2003). Besides, *S. aureus* can adapt and survive in harsh environments, such as drought, cold, and salinity stress (Argudín et al. 2010; Sergelidis et al. 2014). Moreover, it has a strong tolerance for many common bacteriostatic methods, and SFP accounts for a high proportion of bacterial food poisoning (Fischer et al. 2009). Therefore, the research on *S. aureus* has become an important research area in food safety (Xu et al. 2019).

*Staphylococcus aureus* is considered the main pathogenic bacteria in aquatic product processing. Traditional aquatic product processing uses high-salted pickling methods to inhibit the growth of microorganisms in aquatic products and thus prevent corruption in long-term preservation (Fuentes et al. 2010). The existing reports are increasingly concerned about the prevention and treatment of drug-resistant bacteria, which distracts attention from exploring the fundamental mechanism of conferring tolerance to salinity fluctuations (Wong et al. 2019; Ai et al. 2020). Therefore, the molecular mechanism of *S. aureus* for the tolerance of extremely high salt stress was studied. Its tolerance to high salt stress was...
weakened in a targeted way to enhance the inhibitory effect in aquatic products. The control methods of *S. aureus* are important research directions aquatic product processing and have practical significance for the diagnosis of diseases (Price-Whelan et al. 2013). Herein, we studied the molecular mechanism underlying the response of *S. aureus* ZS01 to extremely high salt stress. A targeted weakening of its tolerance to high salt stress can be developed as a new therapy other than antibiotics.

Generally, osmotic stress often has a great effect on the structure, chemistry, and physiology of a bacterial cell. According to the previous studies on bacteria response to salinity stress, biofilm assays showed a positive correlation between biofilm formation and increased concentration of NaCl (Beckingsale 2008). Islam et al. demonstrated that the addition of NaCl increased the production of polysaccharide intercellular adhesin (PIA) to induce changes in biofilm (Islam et al. 2015). Several studies on osmoregulation in *S. aureus* (Graham and Wilkinson 1992; Cebrián et al. 2015), choline, glycine betaine, l-proline, and taurine have demonstrated osmotic protective effect (Vijaranakul et al. 1995). The accumulation of permeating agents can effectively help bacteria to survive in a hyperosmolar environment. For example, *Listeria monocytogenes* respond to a hyperosmolar environment by transporting proteins to absorb osmotic protectants; *Bacillus subtilis* osmotically regulates by the synthesis of betaine (Boch et al. 1994; Angelidis and Smith 2003). It has been reported that *Enterococcus faecalis* can reduce the metabolism of carbohydrates and amino acids and increase the synthesis of nucleotides to adapt to alkaline stress (Ran et al. 2015). There are many reports about the cellular and signaling molecular responses of bacteria to osmotic stress. *Escherichia coli* increased the expression of channel proteins through the EnvZ/OmpR two-component regulatory system to increase tolerance to hyperosmotic environments (Oshima et al. 2002). In *Streptococcus mutans*, ATP-binding cassette (ABC) transporters act as sugar metabolism transporters to resist environmental stress (Nagayama et al. 2014).

Recently, omics research has predominantly been used to explore these biological phenomena. It mainly relies on the rapid development of high-throughput sequencing technology (Xin et al. 2019). The application of these platforms makes outstanding contributions to in-depth research on the occurrence of the mechanisms. With transcriptomics data as the research background, Guan et al. evaluated the role of the *afap* 1 in oxidative stress and aflatoxin synthesis (Guan et al. 2019). Kim et al. explained the thermotolerant mechanism of *E. coli* by using transcriptome and metabolome analyses (Kim et al. 2020). In this study, transcript and metabolite datasets have been combined to reveal the response mechanism via correlation and cluster analyses and further manifested as the connection networks between genes and metabolites. Salt-tolerant strains can evolve specific mechanisms in response to salt stress and changes. This mechanism was studied in our previous report, but it focused on the influence of DEPs at the proteomics level (Ming et al. 2019). An integrated analysis revealed more insights into the salinity stress-responsive genes, DMs, and pathway interactions than a separate analysis. The screening of possible key genes and metabolites provides an opportunity for us to understand how *S. aureus* ZS01 responds to salt stress, helps us to prevent SFB infections, and develops new therapies other than antibiotics.

### Materials and methods

#### Bacterial strain and growth conditions

High salt-tolerant strain *S. aureus* ZS01 was separated from pickled *Bullacta exarata* in Ningbo City. Commercial *S. aureus* ATCC 27217 was purchased from the Institute of Microbiology, Chinese Academy of Sciences. Bacterial strain characteristics and growth conditions were determined using the method previously described by Ming et al. (2019). Briefly, after isolation, identification, and confirmation of salt tolerance, *S. aureus* ZS01 and *S. aureus* ATCC 27217 were incubated for 48 h in a broth medium supplemented with 0%, 10%, and 20% NaCl, respectively. Incubation was at 37 °C with shaking at 150 rpm before harvest. Cell growth was monitored by measuring the optical density at 600 nm on a spectrophotometer. Cell concentrations were determined using the 3 M™ Petrifilm™ Rapid *S. aureus* Count Plate method.

#### Scanning electron microscope (SEM) analysis of morphological observation

Take 1.5 mL of bacteria liquid and centrifuge at 8000×g for 4 min, discard the supernatant. The cells were processed for SEM according to the method described by Kong et al. (2018). The samples were gold-coated with a multifunctional sample surface treatment machine. A Hitachi S3000N was used for the SEM image capture. The magnifications used were ×10,000.

#### Biofilm analysis

Appropriate dilutions were made to obtain a concentration of approximately 5 × 10⁶ CFU/mL. The solution was centrifuged at 8000×g for 2 min to collect the cells. Biofilm was stained with FITC-ConA (4 °C, 30 min) and PI (4 °C, 15 min), then imaged. Biofilm analysis was performed according to the method described by Rodriguez-Melcon et al. (2019). A Zeiss LSM880 confocal laser scanning
microscope (Zeiss LSM880, Germany) was used for the confocal laser scanning microscopy (CLSM) image capture. The Zen black 2.1 software was used to perform image analysis, biofilm thickness measure, and export.

**Total RNA isolation and transcriptome analysis**

Total RNA was extracted using a commercial RNA purification kit (Invitrogen, California, USA), purity and quantity were analyzed using the Agilent 2100 Bioanalyzer, and rRNA was removed using the Ribo-Zero Magnetic kit (G+G-Bacteria) (Epicentre, Wisconsin, USA) according to the manufacturer’s instruction. Sequencing was carried out on the Illumina Hiseq 4000 platform for 2×100 bp/300 bp (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China) (Ran et al. 2015). Each treatment was carried out in duplicate.

The raw sequence data (raw reads) were filtered with the Seq Prep and Sickle software to obtain clean data. The Q20, Q30, GC-content, and sequence duplication levels of the clean data were calculated (Zhang et al. 2017). High-quality reads from each sample were aligned to the reference genome *S. aureus* ATCC 27217 (https://www.ebi.ac.uk/ena/data/view/GCA_000597965) using the Bowtie software (Zhao et al. 2018). Fragments per kilobase of transcript per million fragments represented the expression values of predicted *S. aureus* transcripts (Cole et al. 2010). An adjusted FDR ≤ 0.05 and |log2FC| ≥ 1 were differentially expressed genes (DEGs). Furthermore, enrichment analysis of the DEGs was analyzed via Gene Ontology (GO) enrichment analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis to obtain a detailed description. Differences with a p value of ≤ 0.05 were used as a threshold to determine the significant enrichment.

**Quantitative real-time PCR (qRT-PCR)**

Ten DEGs were selected for qRT-PCR to validate our Illumina sequencing data. Total RNA was extracted using the commercial TransZol Up Plus RNA Kit (TRAN, Beijing, China) according to the manufacturer’s instructions. Reverse transcription was performed using the Prime Script™ RT reagent kit (TRAN, Beijing, China). The primers were designed with the Primer Premier 5.0 software. The sequences of the primer pairs are listed in Table S1. Rotor-Gene 6000 real-time PCR machine (Corbett, Australia) and SYBR® Premix Ex Taq™ II were used for qRT-PCR analysis. The relative gene expression was normalized internally to 16 s rDNA level and quantified according to the 2−ΔΔCt method.

**Metabolite analysis**

The extraction and derivatization of metabolites were performed as previously reported (Ming et al. 2018). Then gas chromatography-mass spectrometry (GC–MS) was used to analyze the metabolites. The column temperature programming was as follows: 90 °C held for 3 min; from 90 to 160 °C at 5 °C/min, held for 0 min; from 160 to 220 °C at 2 °C/min, held for 1 min; from 220 to 290 °C at 10 °C/min, held for 0 min. Each treatment was performed in triplicate. Principal component analysis (PCA) was performed using the SIMCA-P+ ver14 software (Hashim et al. 2014). Pathway analysis was performed with MetaboAnalyst 4.0 (Chong et al. 2019).

**Integrated transcriptome and metabolome analyses**

Spearman correlation test was used to analyze the correlation between candidate gene expression and discriminant metabolite content. Discriminant metabolites contain amino butanoic acid, glycolic acid, d-erythrose, sebacic acid, xylitol, d-threitol, n-hexadecanoic acid, Myo-inositol, heptacosane, undecanedioic acid, t-proline, phosphoric acid, succinate, d-arabinose, and d-mannitol. In addition, the functional annotation of hub genes was analyzed based on the GO database (http://geneontology.org/) and the KEGG database (http://www.kegg.jp/kegg/pathway.html).

**Results**

**High concentrations of NaCl affect cell growth and morphology**

It can be seen from the growth curve (Fig. 1A, B), with the salinity increased, the growth of *S. aureus* was inhibited. The growth activity of the control group was higher than that of the 10% NaCl treatment group. The growth activity of *S. aureus* in the 20% NaCl treatment group did not change, and the cells did not proliferate. Under the stress of 10% NaCl concentration, the growth activity of *S. aureus* ZS01 was significantly higher than that of *S. aureus* ATCC 27217. Therefore, we determined that the tolerance of high salt-tolerant strain *S. aureus* ZS01 to salt stress was higher than standard strain *S. aureus* ATCC 27217. Therefore, we determined that the tolerance of high salt-tolerant strain *S. aureus* ZS01 to salt stress was higher than standard strain *S. aureus* ATCC 27217 under the same inoculum amount.

In the control group, the morphology of *S. aureus* ZS01 was spherical, the surface of the cell was smooth without damage or wrinkles, and the size was relatively uniform (Fig. 1C). With the increase of salt concentration, there was no significant change of cell morphology in the 10% NaCl
treatment group (Fig. 1D). In the 20% NaCl treatment group, the cell membrane ruptured, the cell contents overflowed, and some cells showed shrinkage (Fig. 1E).

From the above results, it can be seen that growth activity inhibition and morphological destruction of *S. aureus* ZS01 in the 20% NaCl treatment group are more obvious than that in the 10% NaCl treatment group. High salinity stress can change the permeability of the cell membrane and rupture the cell membrane, leading to cell death.

**High concentrations of NaCl affect biofilm formation**

The distribution of the bacteria and extracellular polymeric substances (EPS) was observed from 3D views of CLSM images (Fig. 2A–C). FITC-ConA can bind to cell wall polysaccharides to emit green fluorescence, and PI can penetrate bacterial cells and bind to DNA to emit red light. EPS as the main component of biofilm emits green fluorescence; bacterial DNA emits red fluorescence. With the increase of NaCl concentration, the thickness of biofilms increased and then decreased, and the difference between adjacent groups was significant (Fig. 2D). The results show that in the concentration range of less than 10% NaCl, a high concentration of NaCl contributes to the formation of biofilms. When the concentration of NaCl is higher than 10%, NaCl has an inhibitory effect on the formation of biofilm.

**Transcriptomic profiling through RNA-Seq**

The PCA analysis of transcriptomics data revealed that all samples clustered according to different treatments. As shown in Fig. 3A, duplicate samples have certain similarities and obvious differences between different treatments. At the same time, it validated the unique transcriptional reprogramming caused by different salt stress. Compared with the control group, 248 DEGs (121 upregulated and 127 downregulated) and 891 DEGs (365 upregulated and 526 downregulated) were identified in the 10% and 20% NaCl treatment groups, respectively (Fig. 3B, C). Compared with the 10% NaCl treatment group, 1063 DEGs (399 upregulated and 664 downregulated) were identified in the 20% NaCl group (Fig. 3D). Furthermore, the number of downregulated genes was higher than that in the three groups. Comparing Fig. 3B, C, more genes were mobilized to participate in the
process of high salt stress following with the salt concentration increasing.

Hierarchical cluster analysis of the DEGs was conducted using the HemI 1.0 software (Deng et al. 2014). Four expression change patterns were displayed among these DEGs with increasing concentrations of NaCl (Fig. 4). The four patterns are Pattern I (increase/increase); Pattern II (increase/decrease); Pattern III (decrease/decrease) and Pattern IV (decrease/increase) (Table S2). The numbers of DEGs showed each pattern were 2, 80, 43, and 19, respectively. As a result, these DEGs could be considered the candidate target genes for the direct regulation of salt stress. In addition, cluster analysis displayed that these genes were abundant in some pathways related to membrane transport, redox

Fig. 2 CLSM images of S. aureus ZS01 biofilm formation changes. A 0% NaCl group; B 10% NaCl group; C 20% NaCl group; D The thickness of biofilm. S. aureus ZS01 was incubated for 48 h in a broth medium supplemented with 0%, 10%, and 20% NaCl, respectively. The concentration of the collected bacterial solution is $5 \times 10^6$ CFU/mL. Biofilm was stained with FITC-ConA (4 °C, 30 min) and PI (4 °C, 15 min), then imaged. $\lambda_{ex} = 488$ nm, $\lambda_{em} = 520$ nm was used for FITC-ConA detection (green channel), $\lambda_{ex} = 543$ nm, $\lambda_{em} = 572$ nm was used for PI detection (red channel). Three fields of view are randomly selected for each specimen and scanned layer by layer along the Z-axis. CLSM images were captured using a Zeiss LSM880 confocal laser scanning microscope (Zeiss LSM880, Germany) with ×40 objective lens. Z stack represents the thickness of biofilm.

Fig. 3 Principal components analysis (PCA) of transcriptomics data and volcano plot of the genes identified in S. aureus ZS01 treated with different concentrations of NaCl. A PCA of transcriptomics data for each group. Circle represents 0% NaCl group. Square represents 10% NaCl group. Triangle represents 20% NaCl group. B Volcano plot of the genes (0% NaCl vs. 10% NaCl); C Volcano plot of the genes (0% NaCl vs. 20% NaCl); D Volcano plot of the genes (10% NaCl vs. 20% NaCl). Each point represents a gene, and the red and green areas represent upregulated (Log₂FC ≥ 1 and FDR ≤ 0.05) and downregulated (Log₂FC ≤ -1 and FDR ≤ 0.05) genes, respectively.
process, metabolism, transcription factor activity, kinase activity, phosphatase activity, and stress response.

The predicted five KEGG pathways were statistically significant in the transcriptomic profiling (Table S3). For two comparison groups (0% NaCl vs. 20% NaCl and 10% NaCl vs. 20% NaCl), DEGs were enriched in ribosome pathways (45 genes and 51 genes). For the third comparison group (0% NaCl vs. 10% NaCl), DEGs were enriched in glycolysis/gluconeogenesis metabolism (12 genes), pyruvate metabolism (12 genes), and glucagon signaling pathway (4 genes). After transcriptome enrichment analysis, energy metabolism, carbon, and nitrogen metabolism were enriched most.

**Verification by qRT-PCR**

To assess the reliability of our RNA-seq, 10 DEGs were quantified using qRT-PCR. As shown in Fig. 5, the trend in qRT-PCR expression was in agreement with the RNA-seq expression profile. The results showed similar patterns of mRNA abundance in RNA-seq analysis and qRT-PCR.

Therefore, RNA-seq results can reflect the expression of *S. aureus* transcriptome under high salt stress. Transcriptome data can be used for the analysis.

**Metabolomic profiling through GC–MS**

In total, 76 endogenous metabolites were identified in *S. aureus* ZS01. Then, the concentrations of 76 metabolites in these three groups were calculated based on the internal standard peak area (Table S4). Principal component analysis (PCA) is an overall presentation of the distribution of the original data for the samples. As illustrated in Fig. 6A, the main components of the metabolites were located in different quadrants, indicating clear discrimination among the intracellular metabolome in the three groups. To identify the metabolites affected by salt stress, orthogonal partial least squares discriminant analysis (OPLS-DA) was employed on the metabolic profiles. A total of 15 differential metabolites (DMs) were screened under VIP > 1 with $p < 0.05$ as standard. The 0% NaCl vs. 10% NaCl group screened a total of
ten DMs (amino butanoic acid, glycolic acid, d-erythrosuccinate, sebacic acid, xylitol, d-threitol, n-hexadecanoic acid, Myo-inositol, heptacosane, and undecanedioic acid), the 10% NaCl vs. 20% NaCl group screened a total of six DMs (l-proline, phosphoric acid, butanedioic acid, d-arabinose, d-mannitol, and n-hexadecanoic acid), and the 0% NaCl vs. 20% NaCl group screened a total of six DMs (aminobutanoic acid, l-proline, phosphoric acid, butanedioic acid, d-arabinose, and xylitol) (Table S5).

DMs were subjected to the pathway analysis to get the overall view of their contributions. The metabolic pathways related to salt stress responses are shown in Fig. 6B–D. These pathways were mainly involved in inositol phosphate metabolism, sulfur metabolism, and the TCA cycle (Table S6). Therefore, the results suggest that initiating defense (sulfur metabolism), signal response (inositol phosphate metabolism), and energy regulation (TCA cycle) are the key response pathways for *S. aureus* ZS01 to salt stress.

**Integrated analysis of transcriptome and metabolome**

Transcriptome and metabolome data were combined to gain insight into the regulatory network of *S. aureus* ZS01 under salt stress conditions. The transcriptome finally identified 81 related genes and performed Spearman correlation analysis with 15 DMs. Taking $p \leq 0.05$ as the threshold, paired regulatory relationships were plotted (Fig. 7A). A total of 80 nodes were connected in the network, with 236 edges are displayed in the visualization of Cytoscape. According to the edge greater than 7, ten genes (*antB*, *fnbA*, *gale*, *hisD*, *hisG*, *lysC*, *mnhD*, *proP*, *sdrC_D_E*, and *serA*) for salinity stress response were obtained. Furthermore, all of them were present in Pattern III (decrease/decrease) expression change.

A total of 10 hub genes were divided into three categories: 21 biological processes (BP), 13 cellular components (CC), and 12 molecular functions (MF) (Fig. 7B). Most hub genes were predicted to be involved in binding, catalytic activity, cell, cellular process, single-organism process, and metabolic process. Besides, a total of 17 KEGG pathways were found to be annotated with seven hub genes (Fig. 7C). They were mainly involved in metabolic pathways, biosynthesis of amino acids, biosynthesis of secondary metabolites, histidine metabolism, microbial metabolism in diverse environments, glycine, serine, and threonine metabolism. With the increase of salt stress intensity, *S. aureus* ZS01 showed its adaptive changes. These may be reflected in the GO and KEGG annotations of the hub genes. Overall, the integrated multi-omics analysis identified hub genes that were potentially linked to salt stress. They deserve further attention and in-depth functional study and validation for applications.
Discussion

SFP is one of the pathogenic factors in the process of aquatic production and preservation under high salt conditions all over the world (Hennekinne et al. 2012). The salt-tolerant *S. aureus* is the main factor that plagues the quality and safety of aquatic products. However, the current mechanisms of *S. aureus* adaptation to salt stress are still insufficient. Therefore, this study used a variety of omics methods to clarify this mechanism in a comprehensive manner. We observed that *S. aureus* suffered a large difference in response to salinity stress. Phenotypic and physiological adaptation to environmental conditions is driven by changes in gene regulation, metabolite accumulation, and cell signal transduction. We determined that biofilm formation, signal response, transcription factors, and energy metabolism played important roles in the salt stress defensive system of *S. aureus*.

Biofilm formation

Extracellular polymeric substances (EPS) contributed to biofilm stability and adhesion properties. Capsular polysaccharide (CPS) is the main resistant component of EPS and a major virulence factor in *S. aureus* (Lee 1992; Lee et al. 1994). With the help of biofilms, bacteria exhibit more resistance to salinity stress. The changing pattern of biofilm formation after salt stress is the same as the expression change Pattern II (increase/decrease) of the transcriptome. In Pattern II, we found that CPS biosynthesis protein genes, such as *cap5A, cap5B, cap8C, capD, cap8F*, and *cap5D*, were candidate target genes for direct regulation of salt stress. Microbial biofilms can be used as a “protective suit” to protect microorganisms from extreme temperatures, ultraviolet radiation, extreme pH, high salinity, etc. (Yin et al. 2019). The natural ability of microorganisms to deal with harsh environments is attributed...
to their genetic diversity and physiological adaptability. Previous studies have indicated that bacterial EPS can help alleviate salt stress by reducing sodium content (Banerjee et al. 2019). So, we believe that the formation of biofilm and the production of EPS are important strategies for salt-tolerant *S. aureus* to assist in metabolism in response to salt stress. Enhanced production of EPS is the significant protection mechanism of periphytic biofilms against high concentrations of NaCl. At the same time, according to biofilm phenotype changes and differential gene expression, it can be found that the critical point of 10% NaCl is a relatively special state. These results can be used to explain the different mechanisms in *S. aureus* for tolerating ordinary high-salt and extreme high-salt.

**Fig. 7** Integrated analysis of transcriptome and metabolome. **A** The Spearman correlation network reveals the regulatory mechanisms of the salinity stress response mechanisms. Results of the correlation analysis between key candidate genes and discriminant metabolites. Different colors of nodes represent metabolites (blue), genes (yellow), and hub genes (red). Positive correlations are indicated by a red line, and negative correlations are indicated by a green line. The thickness of the line represents the magnitude of the correlation coefficient. **B** GO annotation of hub genes. Different color blocks represent three secondary categories of GO terms. Purple indicates molecular function, Gray indicates cellular component, Green indicates biological process. **C** KEGG function annotation of hub genes. X-axis indicates the number of hub genes annotated, Y-axis indicates the KEGG pathway terms.

**Signal response**

DM Myo-inositol (VIP = 1.31) was downregulated significantly by 0.79-fold in the 10% NaCl group (p < 0.05) compared to the control group. At the same time, inositol phosphate metabolism has the greatest effect on this group. d-Glucose-6-phosphate is the only source of inositol ring (Raboy and Gerbasi 1996). In this study, the downregulation of fructose-1,6-bisphosphatase gene *fbp3* resulted in the decrease of glucose 6 phosphate content, which was the main reason for the downregulation of inositol content. Shao et al. proved that phosphoinositide has a synergistic effect with carbohydrate transporters, and it is closely related to ion transport (Zhang et al. 2016). It can be inferred that...
the reduction of endogenous glucose was the main reason for the downregulation of *S. aureus* ZS01 inositol phosphate metabolism. The functions of phosphoinositide were diverse, involving stress resistance, signal response, conversion between secondary metabolites, DNA repair, and RNA transport (Hanakahi et al. 2000; Bolger et al. 2008). Inositol phosphate metabolism is complicated. It involves the conversion of various inositol phosphate molecules, which perform various functions in metabolic pathways (Fig. 8). Therefore, we speculated that *S. aureus* ZS01 reduced the conversion of functional molecules, and downregulated inositol phosphate metabolism was one of its strategies to adapt to salt stress.

The studies have displayed that osmotic stress can induce the production of a variety of stress proteins, which have the functions such as cell self-protection, nucleic acid repair, abnormal protein degradation, regulation of intracellular and extracellular osmotic pressure (Kiran and Naomi 2009; Vilhelmsson and Miller 2002). General stress protein gene *dps* and universal stress protein gene *uspA-1* and *uspA-2* were upregulated significantly in the 10% NaCl group (*p* < 0.01), while downregulated significantly in the 20% NaCl group (*p* < 0.01). In *S. aureus*, both general stress response and universal stress response are widely conserved responses allowing bacteria to cope with many stressful conditions. Cold shock protein gene (*cspA, cspC*) and heat shock protein gene (*grpE*) showed significant up-down changes during the process of salt stress enhancement. Our results indicate that these DEGs not only respond to temperature but also play an important role in resistance to osmotic stress. They may act as a full range of firefighters and respond to various extreme environmental changes.

**Transcription factors**

A higher abundance of transcription factors (TFs) as key regulators of transcription is important in *S. aureus* responses to salinity stress. Transcriptome analysis revealed that members of > 30 TF families are responsive to salinity stress in candidate genes. Through the analysis of TF genes, the MarR family held the largest number, which reached 9.

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Fig. 8 Relevant metabolic pathways in *S. aureus* ZS01. Red font indicates upregulated genes, and green font indicates downregulated genes. Matched genes include fructose-1,6-bisphosphatase (*fbp3*), fumarate hydratase (*E4.2.1.2*), formate acetyltransferase (*E2.3.1.54*), citrate synthase (*gltA*), isocitrate dehydrogenase (*icd*), 2-oxoglutarate dehydrogenase E1 component (*sucA*), ATP phosphoribosyltransferase (*hisG*) and histidinol dehydrogenase(*hisD*). IF initiation factor, EF elongation factor, RF release factor, PRPP phosphoribosyl pyrophosphate.
Evidence has shown that MarR family transcription regulators are sensors that sense changes in the environment, can bind small molecule compounds, and quickly start adaptive regulation of transcription levels, including controlling the production of virulence factors, responses to antibiotics and oxidative stress, and catabolism of aromatic compounds in the environment (Wei et al. 2007; Deochand and Grove 2017). The GntR family also dominates in number. Previous studies have shown that GntR family TF combines with metabolites through allosteric effects, regulates the expression of genes, such as metabolism, drug resistance, and virulence, and responds quickly to environmental signals (Kunin and Rudy 1991; Rigali et al. 2002). These results indicate that *S. aureus* ZS01 may be resistant to external stress by regulating the expression of certain stress tolerance genes or drug resistance genes. They turn on different regulatory systems through transcriptional regulation so that cells can adapt to changes in the environment.

**Energy metabolism**

Transcriptome enrichment analysis results show that critical pathways are mainly concentrated in energy metabolism, carbon, and nitrogen metabolism. Metabolome enrichment analysis results show that critical pathways are mainly concentrated in several energy metabolic pathways such as fatty acid biosynthesis, fatty acid degradation, TCA cycle, fructose, and mannose metabolism. From the above results, we concluded that the regulation of energy metabolism pathways was an important strategy for *S. aureus* ZS01 to cope with salt stress.

Hub gene *galE* (UDP glucose 4-epimerase) mediates the mutual transformation of glucose and galactose in the mechanism of galactose metabolism (Roodt et al. 2007). It is the key enzyme for the conversion of galactose into glucose 6-phosphate into glycolytic pathway. In *S. aureus* ZS01, *galE* was continuously downregulated. It reduced the supply of glucose 6-phosphate in glycolysis. This mechanism can slow down the rapid growth of *S. aureus* and reduce the consumption of endogenous glucose to maintain survival. Fructose-1,6-bisphosphatase *fbp3* is one of the key rate-limiting enzymes in gluconeogenesis. It plays a major role in regulating glucose content and sugar metabolism (Brown et al. 2009). In this study, *fbp3* was continuously downregulated with the increase of salt stress. Eventually, it led to the downregulation of the gluconeogenesis pathway. In the process of salt stress, *S. aureus* ZS01 reduced energy consumption by reducing the production of endogenous glucose. In the transcriptome, the pyruvate metabolism pathway fumarate hydratase *E4.2.1.2* and formate acetyltransferase *E2.3.1.54* were upregulated, leading to the upregulation of succinate content (Fig. 8). It can be found that high salt stress can promote the accumulation of succinate. The accumulation of this substance can reduce the sensitivity of the bacteria to salt stress (Zhang et al. 2018), thereby improving the ability of the bacteria to respond to salt stress. This result has been verified in the metabolome, the relative content of DM succinate was continuously upregulated, and it participated in the TCA cycle.

ATP is the key energy substance that sustains life activities in the process of microbial metabolism. NADH or FADH$_2$ reacts with oxidized phosphoric acid via electron transfer to produce a large amount of ATP. In the two comparison groups where TCA is active, according to the transcriptome data, there are three common differentially expressed genes in the electron transport chain, *ssuE* (NADH-dependent FMN reductase), *ndhF* (NADH dehydrogenase subunit 5), and *sdhC* (succinate dehydrogenase cytochrome b-558 subunit) expression levels are downregulated. According to the changes in the expression levels of genes related to the electron transport chain, it is speculated that high salt has an inhibitory effect on the electron transport chain of *S. aureus* ZS01. The utilization of ATP also depends on the enzymes of the citrate cycle. As three key rate-limiting enzymes in the TCA metabolic pathway, the expression levels of *gltA* (citrate synthase), *icd* (isocitrate dehydrogenase), and *sucA* (2-oxoglutarate dehydrogenase E1 component) are downregulated. Downregulation of TCA cycle related enzymes in *S. aureus* ZS01 provides evidence for energy regulation to maintain survivable growth under salinity. The TCA overall shows a low expression trend to maintain cell proliferation under high salt stress. As the salt concentration increases, the transcription of the gluconeogenesis decrease, which reduces the intracellular transport efficiency of glucose. The downregulation of these two energy metabolism pathways indicates that the cells are in a low energy metabolism similar to self-protection under high salt stress. In this respect, our results are consistent with the previous studies (Ming et al. 2019, Lee et al. 2013). The previous studies have shown that self-protection mechanisms can protect cells from hypertonic environments, reduce unnecessary energy loss, and help them focus on survival (Lee et al. 2013). Although stronger stress can hinder cell development and biomass, the life cycle is maintained, suggesting an adapted metabolism can counteract this stress (Arbelet-Bonmin et al. 2020). We conclude that low energy metabolism was an important way to self-protect and overcome high salt stress for *S. aureus* ZS01.

**Amino acids**

Histidine biosynthesis plays an important role in cell metabolism, related to nitrogen metabolism and de novo purine synthesis. This interconnection is due to the reaction catalyzed by the products of *his* gene cluster (Chioccioli et al. 2020). In this study, hub gene *hisG* (ATP
phosphoribosyltransferase) and hisD (histidinol dehydrogenase) were involved in the first and last two steps of the histidine synthesis pathway (Fig. 8), respectively. They led the downregulation of the histidine synthesis pathway. Histidine, as a glycogen amino acid, enters the gluconeogenesis pathway under unfavorable conditions (John 2005). Therefore, in our results, S. aureus ZS01 reduced the production of endogenous glucose by continuously down-regulating histidine synthesis. This is consistent with our results above.

It has been reported that S. aureus will accumulate a large amount of l-proline after salt stress. The accumulation of this amino acid plays a key role in regulating osmotic pressure, protecting cell membranes, cytoplasmic enzymes, and stabilizing proteins (Miller et al. 1991; Townsend and Wilkinson 1992). Therefore, the accumulation of l-proline is used as one of the indicators to measure its salt tolerance. In this study, the synthesis of DM l-proline increased gradually under salt stress. It can be seen that the synthetic osmotic regulator l-proline is one of the important mechanisms for S. aureus ZS01 to resist external salt stress.

Conclusion

Staphylococcus aureus has an extraordinary ability to deal with a wide range of salinity changes. The exhaustive profiling of genes, metabolites, and pathways revealed important information regarding the regulatory networks underlying bacteria responses to salt stress. Herein, we confirmed that biofilm formation, signal response, transcription factors, energy metabolism, and amino acids played important roles in the salt stress defensive system of S. aureus ZS01. Low energy metabolism is the most important self-protection strategy for S. aureus to cope with salinity stress. We can selectively screen potential targets for salt tolerance and pathogenicity of S. aureus and suppress the pathogenicity and toxicity, helping us stop SFB infections. Based on the analysis of omics data, this study provides new insight into selected potential targets for salt tolerance and pathogenicity of S. aureus in aquatic production and preservation.

Supplementary Information

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Author contributions RXW, XRS, CYL, and JZ conceived and designed the experiments. YF, WZY, DZG, and JFF carried out the experiments and data analysis. RXW, XRS, YF, and CYL were involved in drafting the manuscript. All the authors read and approved the final manuscript.

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Data availability The raw sequencing data were deposited at the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under Bio project No. PRJNA704096.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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