

Transcriptomics Analysis of Formic Acid Stress Response in *Saccharomyces Cerevisiae*

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Abstract

Formic acid is a representative small molecule acid cell inhibitor in lignocellulosic hydrolysate, which can inhibit the growth of yeast cells in the process of alcohol fermentation. However, the mechanism of formic acid cytotoxicity remains largely unknown. This study aimed to study the cytotoxicity of formic acid stress to *Saccharomyces cerevisiae*. We evaluated the effects of formic acid on growth metabolism and cell morphology of yeast cells, and comprehensively and systematically analyzed the molecular mechanism of formic acid stress tolerance through transcriptome technology. The results showed that when the concentration of formic acid was 1.8 g/L, the growth of yeast cells was significantly inhibited, the cell surface was wrinkled, and the adhesion between cells was observed, and the cell wall and cell membrane of yeast were destroyed by changing the structure of proteins and carbohydrates, resulting in cell damage. Transcriptome sequencing results showed that formic acid stress inhibited protein biosynthesis, induced oxidative stress, resulted in autophagy, impaired intracellular ATP production and increased consumption, and then impaired normal physiological and metabolic functions of cells. Yeast cells provide sufficient ATP by accelerating glucose metabolism, enhancing electron transport and ATP synthesis more energy to resist formic acid stress, and reduce the expression of genes related to energy metabolism such as intracellular amino acids to achieve an energy-saving strategy. In addition, it can also induce sexual reproduction and spore formation to improve cell tolerance to formic acid. This study initially revealed the molecular response mechanism of *S. cerevisiae* under formic acid stress, and provided a scientific basis for further research on methods to improve the tolerance of cell inhibitors in lignocellulose hydrolysate.

Introduction

Lignocellulose is one of the most abundant clean and renewable resources in nature. Its hydrolysate can be fermented by *Saccharomyces cerevisiae* to produce cellulosic ethanol, which can effectively alleviate the urgent energy crisis and environmental pollution problems (Auesukaree et al. 2017; Mosier et al. 2005). However, toxic compounds are often introduced during the pretreatment and hydrolysis of lignocellulosic raw materials. According to the sources of these toxic compounds, they can be divided into three categories: weak acids (Martani et al. 2014; Matsushika et al. 2012), furans and phenols (Almeida et al. 2007; Sasano et al 2012). These toxins can reduce cell growth and have negative effects on the performance of fermentation microorganisms. Specifically, formic acid and acetic acid are common weak acid inhibitors in lignocellulosic hydrolysates, which are mainly produced by the degradation of 5-hydroxymethylfurfural (Larsson et al.1999), and a part of formic acid is produced by the degradation of furfural under acidic conditions (Zhang et al. 2012). Acetic acid is mainly produced in the process of deacetylation of hemicellulose, and its concentration is usually between 1–15 g/L (Wende et al. 1999). The concentration of formic acid is usually lower than that of acetic acid, but it is more toxic to *S. cerevisiae* than acetic acid (García-Aparicio et al. 2006; Martin et al. 2007). The concentration of other toxic and weak acid hydrolysates is rarely reported, and its concentration is even lower than that of formic acid. Although it is not easy to elucidate the inhibitory mechanism of weak acids, it has been

suggested that the inhibitory effect of weak acids is due to uncoupling theory and intracellular anion accumulation (Palmqvist et al. 2000; Pampulha et al. 2000). The undissociated weak acid molecules can pass through the cell membrane and enter the cell, and dissociate into protons and acid radical ions in the cell, thereby causing intracellular acidification. This acidification may cause the intracellular pH to drop and inhibit cell growth and product formation, and thus affect cell metabolism (Lohmeier-Vogel et al. 1998; Guldfeldt et al. 1998). Intracellular anions believe that only the anion form of weak acids can accumulate in the cell, thus causing the toxicity of weak acids (Helle et al. 2004; Russel et al. 1992) and resulting in the degradation of vacuoles (Suzuki et al. 2012).

In recent years, RNA sequencing, as a revolutionary tool for transcriptomics analysis through deep sequencing technology, has been successfully applied to *S. cerevisiae* (Nagalakshmi et al. 2008). Abbott et al. (2009) research found that overexpression of *CTT1* encoding cytoplasmic catalase increased the specific growth rate of yeast under acetic acid treatment at pH 3. The Mira study found that multiple genes in yeast cells are involved in the toxic effects of acetic acid. These genes are mainly involved in transcription, cell wall integrity, intracellular pH homeostasis, carbohydrate metabolism, and cell absorption of nutrients (Mira et al. 2010). However, as far as we know, the current tolerance mechanism of *S. cerevisiae* in response to formic acid stress needs to be further elucidated at the transcriptome level. In this study, in order to explore the molecular toxicity mechanism of formic acid on *S. cerevisiae*, and combined with scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR) analysis. Transcriptome-based high-throughput sequencing technology was used to study the formic acid stress response and tolerance mechanism of *S. cerevisiae*, in order to initially reveal the molecular response mechanism of *S. cerevisiae* under formic acid stress at the molecular level, and provide a theoretical basis for further improving the tolerance of *S. cerevisiae* lignocellulose hydrolysate inhibitors.

Materials And Methods

Strain culture

The *S. cerevisiae* GGSF16 strain used in this study was isolated and preserved by the Microbiology Laboratory of Guangxi University of Science and Technology. The strain was stored on YPD medium plates (yeast extract 10 g/L, peptone 20 g/L, glucose 20 g/L, agar powder 20 g/L). Activate and culture for 14 hours in a shaker at 30°C and 150 r/min. Inoculate the activated GGSF16 yeast cells in a fresh YPD medium at 30°C with an initial yeast cell number of 4.2×10^7 CFU/mL. After culturing for 6 hours, report to the experimental group. Add an appropriate amount of formic acid to the culture medium to make the final concentration 1.2 g/L, 1.5 g/L, 1.8 g/L, 2.1 g/L, and use the bacterial solution without formic acid as the control group. Then cultivated for 24 hours and monitored at 600 nm (OD_{600}) with an ultraviolet spectrophotometer UV2000. Three biological replicates were performed. Then we select 1.8 g/L formic acid for subsequent experiments according to the growth state of *S. cerevisiae* GGSF16. To repeat the above culture steps, the group with 1.8 g/L formic acid as treatment group (for1, for2, for3) and the non-treated group (ck1, ck2, ck3) as the control group. and the yeast cells were collected for RNA extraction after continuous culture for 2 hours.

SEM analysis

After treatment with formic acid, yeast cells were centrifuged at 8000 r/min 4 °C for 10 min to collect, washed with PBS buffer for 3 times, and the bacteria were fixed in 2.5% glutaraldehyde, fixed overnight at 4°C, centrifuged at 8000 r/min 4 °C for 10 min to collect the bacteria. 20%, 50%, 70%, 80% and 100% ethanol were washed successively, each concentration was treated for 10min, and the bacteria were collected by centrifugation at 8000 r/min at 4 °C for 10min. Finally, the yeast cells were dried, and after spraying gold, the yeast cells were observed and photographed using the FehnPhenom scanning electron microscope.

FTIR analysis

Collect the yeast cells by centrifugation, wash the cells 3 times with PBS, and freeze-dry. This experiment uses the potassium bromide tableting method (Rogowski et al. 2018), and the freeze-dried yeast cells and potassium bromide are in a mass ratio of 1:20 Mix and grind into a uniform powder with an agate mortar. The scanning conditions were set as follows: the spectral range was $400\text{cm}^{-1}\sim 4000\text{cm}^{-1}$, the resolution was 4cm^{-1} , potassium bromide was used as a blank control, and each sample was repeated 3 times under the same conditions.

RNA extraction, library construction and sequencing

Samples containing yeast cells were centrifuged at $12000\times g$ at 4 °C for 2 min. After turbid supernatant was removed by decantation, yeast cells were recovered from the pellets and stored at -80 °C. Total RNA was extracted using the yeast RNA extraction kit. The extracted total RNA sample is subject to a simple quality test before it can be used for subsequent transcriptome analysis, including the detection of RNA integrity, concentration, and protein contamination to check whether they are qualified. After the total RNA detection was qualified, the mRNA was enriched with magnetic beads with Oligo (dT) and fragments were added with an appropriate amount of interrupting reagent. The cDNA library was constructed using the mRNA as template and was sequenced using the BGISEQ sequencing platform at last. The library was tested and sequenced were completed by Shenzhen BGI Technology Services Co., Ltd.

Reads mapping, annotation and analysis

Quality control was carried out on Raw Reads obtained by sequencing to obtain high-quality Clead Reads. First, the Reads containing joints and the Reads with high and low quality of unknown base N content should be removed to obtain Clead Reads, which were used as data for subsequent experimental studies. Sequence alignment analysis was performed between Clead reads and the specified reference genome using HISAT, and differential expression analysis was performed between the two sample groups using DESeq2 algorithm. Screen differentially expressed genes (DEGs) by $q\text{value}<0.05$ and $\text{Fold Change}\geq 2$. The GO and KEGG pathway enrichment analysis of DEGs were performed using David (<https://david.ncifcrf.gov/>) and Kobas (<http://kobas.cbi.pku.edu.cn/>) database.

Quantitative real-time PCR analysis

Totally, twelve representative genes were selected to evaluate the validity of RNA-seq by real-time PCR. The Revertaid First Strand cDNA Synthesis Kit from Thermo Fisher Scientific was used to remove genomic DNA and reverse transcribed RNA to synthesize cDNA. qRT-PCR was performed using Fluorecent Quantitative PCR Detection system Line Gene 4800 (FQD-48A, HANGZHOU BIOR TECHNOLOGY CO., LTD). The PCR reaction conditions were as follows: pre-denaturation at 95 °C for 2 min, denaturation at 95 °C for 15 s, annealing at 55 °C for 15 s, and extension at 72 °C for 1min, with a total of 40 cycles. All reactions were performed in triplicate, The relative expression level of target genes was measured with the $2^{-\Delta\Delta CT}$ method and ACT1 was used as the reference gene.

Results

The effects of different concentrations of formic acid on the growth of *S. cerevisiae* GGSF16 cells

The cell growth of GGSF16 under different concentrations of formic acid stress (Fig. 1). The growth inhibition of formic acid on *S. cerevisiae* increased with the increase of formic acid mass concentration. We can see that there was little effect on the growth of GGSF16 when exposed to 1.2 g/L formic acid. the growth of GGSF16 was immediately inhibited under the stress of adding 1.5 g/L formic acid, but as time does on, the inhibition gradually weakened, and the final growth rate was slightly lower than that of the formic acid-free treatment group. When the mass concentration of formic acid was increased from 1.5 g/L to 1.8 g/L, the growth of yeast cells was significantly inhibited, and the stabilization time was prolonged compared with the formic acid-free treatment group. When exposed to 2.1 g/L formic acid stress group, the GGSF16 cells hardly grew. In order to obtain valuable transcriptome data, the GGSF16 cells exposed to 1.8 g/L formic acid stress was selected as the experimental group in this study.

The effects of formic acid on the morphology of yeast cells

The morphology of yeast cells in the control and formic acid treatment groups was significantly different (Fig. 2). In the formic acid treatment group (Fig. 2B), the surface of yeast cells was obviously deformed, the surface of cell membrane became rough, and the edge of cell wall collapsed. While in the control group (Fig. 2A), the cell surface was smooth and full, and the appearance was oval or round, indicating that formic acid had obvious damage to the cell wall and cell membrane of *S. cerevisiae*, causing the cells lose the protection of cell wall, causing the normal physiological metabolism of cells to be blocked, and it was difficult for nutrients to enter and exit the cells, leading to cell damage and death.

FTIR analysis

FTIR was a structure analysis technology based on the vibration of functional groups and polar bonds in compounds. It was characterized by simple operation, fast speed and high sensitivity. In recent years, it has been widely used in the structure analysis of macromolecular compounds and the analysis of the secondary structure of proteins. and it is a powerful tool for molecular structure information (Yu et al.

2019; Bosch et al. 2006). Studies have shown that yeast cell wall is mainly composed of β -glucan, mannoglycoprotein, a small amount of chitin and lipids, which plays an important role in maintaining cell morphology and inter-cell recognition (Galichet et al. 2001). These substances can provide a large number of active groups, and the stretching, bending and deformation vibrations generated by these groups can generate obvious absorption peaks in the infrared spectrum. In the spectrogram of yeast, the most important band to characterize protein is the amide band, and the amide I band at 1650 cm^{-1} is formed by the stretching vibration of C=O and the bending vibration of N-H. which indicates that the secondary structure of protein in yeast is dominated by α helix (Piotrowska et al. 2010). The amide II band at 1540 cm^{-1} is caused by the bending vibration of N-H and the stretching vibration of C-N. The amide III band at 1240 cm^{-1} may be caused by the bending vibration of C-N, the stretching vibration of C-O in -COOH, and the deformation vibration of P=O, which represents the asymmetric stretching of the phosphate diester bond and is related to the phospholipid bilayer (Salman et al. 2019). The absorption peaks at about 1080 cm^{-1} and 915 cm^{-1} represent the carbohydrate and polysaccharide ring bonds in the yeast RNA, DNA or cell wall, respectively (Wang et al. 2017). The absorption peak located at 3307 cm^{-1} is caused by the stretching vibration of -OH in chitin and -NH in secondary amines, while the absorption peak located at $3100\sim 2800\text{ cm}^{-1}$ is due to the antisymmetric vibration of -CH group in fatty acids, representing lipid functional groups (Iqbal et al. 2009).

Protein is the main component of cell membrane and cell wall. Red shift of amide I band ($1651.3/1650.3\text{ cm}^{-1}$) and blue shift of amide side band ($1542.9/1545.4\text{ cm}^{-1}$) were observed after formic acid treatment, indicating that oxygen and nitrogen atoms on protein peptide chain may have corresponding changes, suggesting that formic acid stress may cause protein denaturation in yeast cells (Fig. 3). In addition, the peak of the amide III band ($1243.3/1242.4\text{ cm}^{-1}$) produced red shift, which may be caused by the phospholipid bilayer damaged by formic acid treatment, and the permeability of the cell membrane was changed and some nucleic acids and proteins were released. The peak sites were significantly increased at 3307.7 cm^{-1} , 3002.6 cm^{-1} and 2928.6 cm^{-1} , indicating that formic acid altered the structure of chitin in the cell wall and damaged the lipid of yeast cell membrane. The peak at 914.2 cm^{-1} was enhanced, indicating that formic acid changed the polysaccharide hydroxyl skeleton on the surface of *S. cerevisiae*, which in turn changed the structure of the yeast cell wall. Therefore, it is speculated that formic acid damages the yeast cell wall and membrane by changing the structure of proteins, lipids and chitin, leading to cell death.

Transcriptome data analysis

The original data was processed through a series of data processing to filter impurities to get filtered data. The average clean read of each sample reaches 6.41 Gb, and the average Q20 of the two groups were 97.27% and 97.26%, and the Q30 averages are 89.73% and 89.53%. High-quality reads were compared with the designated reference genome using Hisat software, and the results showed that more than 95.91% and 96.31% reads of the two groups covered the reference genome, and the quality of the original sequencing was qualified and could be used for subsequent data analysis.

Screening of differentially expressed genes

In order to study the differentially expressed genes before and after the addition of formic acid, differential expression identified was conducted for all genes obtained after sequencing between the two groups. With Fold Change > 2 and $q\text{value} < 0.05$ as the identified conditions, A total of 1504 DEGs were identified, of which 797 were up-regulated genes and 707 were down-regulated genes (Fig. 4). Each dot in the figure represents a gene, The red ones are up-regulated gene, the green ones are down-regulated gene, and blue ones are that is not significantly different.

GO enrichment analysis

GO enrichment analysis can provide three types of descriptions for gene products, namely Biological Process (BP), Cellular Component (CC) and Molecular Function (Molecular Function, MF). Through the GO enrichment analysis of differential genes, The function of genes can be studied (Fig. 5). Through GO enrichment, we found that 8 of 25 significantly enriched pathways were related to membrane transport function, including fructose transmembrane transport, mannose transmembrane transport, glucose transmembrane transport, fructose transmembrane transporter activity, transporter activity. These DEGs changed differently under formic acid stress, suggesting that formic acid affected the function of cell membrane and membrane transporter. In addition, most DEGs are enriched in ribosome metabolic pathways, indicating that formic acid has a certain impact on ribosomes. It can be seen from the scanning electron microscope that the surface morphology of the yeast cells is greatly changed after formic acid treatment, indicating that the cell membrane and cell wall are damaged, so that the cell loses the protection of the cell wall, causing the normal physiological metabolism of the cell to be blocked, and it is difficult for substances to enter and exit the cell, resulting in poor cell growth or death. The infrared analysis results show that formic acid destroys yeast cell walls and cell membranes by changing the structure of proteins, polysaccharides, lipids and chitin, which is consistent with the changes in cell morphology observed in the electron microscope. GO enrichment analysis showed that under formic acid stress, yeast cells deal with the damage of formic acid to the cell membrane and cell wall by regulating the expression of genes related to membrane transport.

KEGG pathway analysis

The KEGG enrichment analysis of DEGs between the control group and the formic acid treatment group is shown in Fig. 6. It is important to note that there are 115 DEGs are enriched in the ribosome pathways, and 27 DEGs are enriched in the ribosomal biogenesis of eukaryotes, indicating that ribosomes play an important role under the formic acid stress, and found that most genes related to body biosynthesis were down-regulated, while genes related to mitochondrial ribosome biosynthesis were up-regulated. It was also found that 26 DEGs were enriched in glycolysis/gluconeogenesis, 20 DEGs were enriched in starch and sucrose metabolism, 13 DEGs were enriched in TCA cycle, 12 DEGs were enriched in galactose metabolism, 10 DEGs were enriched in fructose and mannose metabolism, 38 DEGs were enriched in meiotic yeast, 40 DEGs were enriched in MAPK signaling pathway, It was speculated that *S. cerevisiae* accelerates glucose metabolism through glucose transmembrane transport, thus synthesizing a large

amount of ATP, providing sufficient energy for resisting formic acid stress, which is consistent with the GO enrichment analysis results. In addition, it is possible to enter sexual reproduction and spore formation to improve offspring adaptation to the environment, thereby enhancing formic acid tolerance. Interestingly, we also found that the differential genes involved in cysteine and methionine metabolism and purine metabolism are also significantly expressed, indicating that amino acid metabolism and purine metabolism also play a role in formic acid stress.

RNA-seq expression validation by quantitative real-time PCR

In order to quantitatively determine the reliability of the transcriptome results, we detected the expression of 12 candidate DEGs by RT-qPCR. These candidate genes include 10 up-regulated genes and 2 down-regulated genes (Fig.7). The RT-qPCR results showed that, after the addition of formic acid, the expression of *HXK2* and *ENO2*, which were involved in glycolysis pathway, *IMA3* involved in galactose metabolism and *SOR1* and *DSF1* involved in fructose and mannose metabolism, *HSP30* involved in protein folding, *GND2* is involved in pentose phosphate pathway, *ATP14*, *ATP19* and *COX6*, which were involved in ATP biosynthesis were up-regulated, while *PRS3* and *ADE5,7* were involved in purine metabolism were all down-regulated. A high consistency was displayed between the RNA-seq and RT-qPCR data, proving the validity of RNA-seq data for genes with distinct transcript abundance.

Discussions

Ribosome

The ribosome is an important organelle in eukaryotic cells, which is mainly involved in protein biosynthesis in cells. It consists of a 40S subunit, a 60S subunit and 4 types of RNA (Horsey et al. 2004). RNA-Seq analysis showed that under formic acid stress, our research found that most of the DEGs related to ribosomal and ribosomal biogenesis in eukaryotic pathways were significantly down-regulated (Table 1). The expression of genes related to mitochondrial ribosomal proteins is up-regulated, which means that even if formic acid inhibits the biosynthesis of yeast cytoplasmic ribosomes, cells can still use mitochondrial ribosomes to synthesize other proteins to resist the stress of formic acid. Ribosomal protein is an important component of ribosomes. Studies have found that ribosomal protein is not only involved in protein synthesis, but also involved in cell differentiation, cell development regulation, DNA repair and other processes. It plays a crucial role in the growth and development of eukaryotes (Tedesco et al. 2014). In yeast cells, ribosome biosynthesis is highly conserved. It starts from the precursor rRNA in the cytoplasm and ends with the synthesis of mature 40S and 60S ribosomal subunits. In addition, the ribosome synthesizes cells through ribosomes. The required protein can consume about 2/3 of the energy. Li et al. (2010) showed that the expression of genes related to ribosome synthesis was down-regulated under acetic acid stress, indicating that formic acid also had an inhibitory effect on ribosome synthesis in yeast cells. In addition, we also found that the expression levels of differentially expressed genes involved in ribosomal biosynthesis in eukaryotes are also down-regulated. *UTP14*, *UTP13*, *UTP21*, and *UTP5* are components of complexes that have the activity of processing precursor rRNA and

participate in the process. The synthesis of 18S rRNA in the nucleolus and the assembly process of small ribosomal subunits (Dragon et al. 2002), the down-regulation of its expression indicates that formic acid stress may inhibit the biosynthesis of the yeast cell polypeptide chain. Transcriptome analysis shows that formic acid significantly reduces the expression of genes involved in protein and RNA synthesis in yeast cells, indicating that formic acid affects the biosynthesis of yeast cells. Cells allocate the energy they produce reasonably and synthesize materials needed to relieve stress, To reduce the synthesis of some ribosomes and degrade non-essential substances, thereby improving the resistance to formic acid.

Table1 Differentially expressed genes related to ribosome biosynthesis under formic acid stress

Gene ID	Gene Name	Log2 (fold change)	Description
YDR337W	MRPS28	1.22	Mitochondrial ribosomal protein
YPL173W	MRPL40	1.01	Mitochondrial ribosomal protein
YHR147C	MRPL6	1.00	Mitochondrial ribosomal protein
YOR063W	RPL3	-2.93	Ribosomal 60S subunit
YGL076C	RPL7A	-2.61	Ribosomal 60S subunit
YPL131W	RPL5	-2.45	Ribosomal 60S subunit
YLR029C	RPL15A	-2.41	Ribosomal 60S subunit
YJL177W	RPL17B	-2.39	Ribosomal 60S subunit
YOL120C	RPL18A	-2.24	Ribosomal 60S subunit
YPL198W	RPL7B	-2.23	Ribosomal 60S subunit
YGL103W	RPL28	-2.23	Ribosomal 60S subunit
YOL127W	RPL25	-2.20	Ribosomal 60S subunit
YBR031W	RPL4A	-2.20	Ribosomal 60S subunit
YGL031C	RPL24A	-2.06	Ribosomal 60S subunit
RPL13A	RPL13A	-2.03	Ribosomal 60S subunit
YNL178W	RPS3	-2.69	Ribosomal 40S subunit
YDR447C	RPS17B	-2.46	Ribosomal 40S subunit
YOR096W	RPS7A	-2.28	Ribosomal 40S subunit
YJR123W	RPS5	-2.04	Ribosomal 40S subunit
YHR021C	RPS27B	-2.06	Ribosomal 40S subunit
YDL083C	RPS16B	-2.05	Ribosomal 40S subunit
YOL039W	RPP2A	-2.48	Ribosomal protein P2 alpha
YDR382W	RPP2B	-2.08	Ribosomal protein P2 beta
YDL081C	RPP1A	-2.05	Ribosomal stalk protein P1 alpha
YER006W	NUG1	-2.48	GTPase
YNR053C	NOG2	-1.51	GTPase
YLR197W	NOP56	-2.14	Nucleolar protein
YOR310C	NOP58	-2.04	Nucleolar protein

YML093W	UTP14	-1.93	SSU processome complex
YLR409C	UTP21	-1.66	SSU processome complexes
YDR398W	UTP5	-1.64	SSU processome complex
YHR170W	NMD3	-1.72	Ribosomal subunit
YLR222C	UTP13	-1.67	Nucleolar protein

Protein degradation and autophagy

The endoplasmic reticulum is an important organelle in yeast cells and has many general functions, including folding and transport of synthetic proteins. It is the center of various chaperone proteins and enzymes in eukaryotes. Chemical stress will destroy the conformation of the protein, leading to the unfolding and aggregation of the protein (Goldberg et al. 2003). Small HSPs act as chaperones to help fold or refold newborn or denatured proteins and enzymes to maintain functional conformation (Burnie et al. 2006). Interestingly, we found that the expression levels of genes involved in protein folding and degradation were significantly up-regulated under formic acid stress (Table 2). *SSA4*, *HSP30*, and *HSP26* encoding chaperone proteins were significantly induced to resist the stress damage of formic acid to the protein. Ma et al. (2010) found that the deletion mutation of *SSA4* exhibits a significantly longer lag period under HMF stress, indicating that *SSA4* plays an important role in the adaptation and tolerance of HMF. Piper et al. (2015) found in the study of the ATPase activity of yeast cell membranes that *HSP30* has an important effect on maintaining the activity of cell ATPase. It can block the activity of Pma1H-ATPase and ensure sufficient ATP environment in the cell. *FES1*, *SSA3*, *SSA2*, *SSA1* have been identified as chaperones of the HSP70 family, which can help the correctly folding of newly translated proteins, prevent the aggregation of denatured proteins, degrade misfolded proteins and play an anti-apoptotic role in cells (Danial et al. 2004). *EMP46*, *DER1* are involved in the output of misfolded or unassembled proteins and transport the abnormal proteins to the degradation system.

Autophagy was affected by a variety of stress factors, such as energy stress (Meijer et al. 2011), oxidative stress (Wu et al. 2009), starvation (Hailey et al. 2010) and endoplasmic reticulum stress (Qin et al. 2010). It has been proved that autophagy plays an important role in cell survival under adverse conditions, including removal of degradation products, removal of macromolecules and organelles (Mizushima et al. 2007). In this study, autophagy-related genes were significantly up-regulated under formic acid stress (Table 2). *ATG9* encodes a transmembrane protein that participates in the formation of autophagy vesicles and cytoplasmic-cytoplasmic (Cvt) pathways. *ATG7* and *ATG13* encode autophagy-related proteins, which participate in the formation of vesicles in the process of autophagy, and regulate the binding of *Atg12p*, *Atg5p*, *Atg8p* and phosphatidylethanolamine. *ATG2* is involved in autophagy repair. The peripheral membrane protein encoded by *ATG2* is involved in the Cvt pathway and the entry of autophagic vesicles. It is a necessary condition for the formation of isolated cytoplasmic vesicles. *Vps34p* and *vps15p* jointly encode the autophagy of the phosphatidylinositol 3-kinase complex, which is a necessary condition for the formation of autophagy vesicles. Studies have shown that autophagy is an important way for cells to get rid of oxidative stress and is the second level of self-protection against

environmental oxidative stress(Jain et al. 2010). In the absence of autophagy, environmental stress induces the accumulation of intracellular reactive oxygen species (Tal et al. 2009). Interestingly, the expression levels of autophagy related genes were significantly increased under formic acid stress, suggesting that autophagy plays an important role in the response of *S. cerevisiae* to formic acid stress. Therefore, it is speculated that formic acid stress induces oxidative stress through the accumulation of reactive oxygen species in yeast cells, which induces autophagy in yeast cells, and then regulates protein degradation and autophagy genes, making *S. cerevisiae* resistant to formic acid stress. By improving the activity of molecular chaperones, degrading misfolded proteins, reducing misfolded stress, providing raw materials for the synthesis of new proteins, and helping cells to restore normal physiological and metabolic functions.

Table 2 Differentially expressed genes related to protein degradation and autophagy under formic acid stress

Gene ID	Gene Name	Log2 (fold change)	Description
YER103W	SSA4	3.62	Heat shock protein 70 family
YCR021C	HSP30	3.08	Stress-responsive protein
YBR072W	HSP26	2.13	Small heat shock protein with chaperone activity
YBR101C	FES1	2.11	Hsp70 nucleotide exchange factor
YBL075C	SSA3	1.98	Heat shock protein 70 family
YDR258C	HSP78	1.78	Oligomeric mitochondrial matrix chaperone
YLL026W	HSP104	1.68	Heat shock protein
YLL024C	SSA2	1.47	HSP70 family ATP-binding protein
YAL005C	SSA1	1.12	Heat shock protein 70 family
YLR080W	EMP46	1.64	ER-derived COPII-coated vesicles
YBR201W	DER1	1.04	ER membrane protein
YNL242W	ATG2	2.00	Participate in the recovery of autophagy
YHR171W	ATG7	1.77	Participate in vesicle expansion and formation
YDL149W	ATG9	1.56	Involved in autophagic vesicle formation
YPR185W	ATG13	1.50	Involved in vesicle formation during autophagy
YBR097W	VPS15	1.61	Phosphatidylinositol (PI) 3-kinase
YLR240W	VPS34	1.22	Phosphatidylinositol (PI) 3-kinase
YNL006W	LST8	1.19	Proteins required for Gap1p transport

Mitochondrial function and energy metabolism

Mitochondria, as an energy center, drive a large number of energy-needed processes in cells, and the expression of differential genes involved in this process are up-regulated (Table 3). *COX17*, *COX10*, *COX8* and *COX6* are not only related to mitochondrial respiratory chain complexes III, IV, mitochondrial electron transport and oxidative phosphorylation, but also encode cytochrome oxidase (COX). which catalyzes electron transfer from cytochrome C to molecular oxygen and catalyzes proton pump across the mitochondrial inner membrane to produce large amounts of ATP. Interestingly, the expression of energy-related genes *ATP19*, *ATP14*, *ATP20*, *ATP4* and *PPA2* increased significantly, which played an important role in ATP synthesis. Our study also found that the expression level of *PMA2* a gene regulating plasma membrane H⁺-ATPase activity, was also significantly up-regulated, which could pump protons out of cells and regulate cytoplasmic pH and plasma membrane potential. Yeast cells will choose to enhance the expression of genes related to electron transport and ATP synthesis, Expression provides sufficient energy for H⁺ and acid radical ion transport, maintain the stability of intracellular pH, and thus improve the tolerance of yeast cells to formic acid.

Interestingly, genes involved in starch and sucrose metabolism, such as *GSY1*, *GSY2*, *GLG1*, *GLG2*, *GPH1*, *PGM2*, genes involved in fructose and mannose metabolism, such as *PFK27*, *SOR1*, *DSF1*, *MAN2*, *DAK2*, genes involved in galactose metabolism, such as *IMA1*, *IMA2*, *IMA3*, *GAL7*, *GAL2*, *MPH3*, were significantly up-regulated under formic acid stress (Table 3). *GSY1* and *GSY2* encode glycogen synthase, which catalyzes glycogen synthesis. When yeast cells are undernourished by environmental stress, they synthesize glycogen to provide energy metabolism and adjust the energy demand for biosynthesis. *GPH1* encodes glycogen phosphorylase, which can form glucose-1-phosphate catalyzed by glycogen and further convert glucose-6-phosphate catalyzed by glucose phosphate mutant enzyme. This is a key step in hexose metabolism. The up-regulation of the gene expression accelerates the conversion of glucose-1-phosphate to glucose-6-phosphate. *SOR1*, *DSF1* and *MAN2* encode sorbitol dehydrogenase and mannitol dehydrogenase, respectively, providing fructose-6-phosphate for EMP pathway, thereby accelerating ATP generation. *IMA1*, *IMA2*, *IMA3*, and *GAL7* are involved in the decomposition of maltose, and *GAL2*, *MPH3*, *MPH2*, and *MAL31* are involved in the transport of galactose and maltose. These genes related to energy metabolism were significantly up-regulated in the response of *S. cerevisiae* to formic acid stress. It is speculated that when yeast cells are subjected to formic acid stress, certain energy is stored to resist damage, which indicates that energy metabolism is very important in the response to formic acid stress in yeast cells.

Table 3 Differentially expressed genes related to mitochondria and energy metabolism under formic acid stress

Gene ID	Gene Name	Log2 (fold change)	Description
YLL009C	COX17	1.93	Cytochrome c oxidase
YPL172C	COX10	1.10	Cytochrome c oxidase
YLR395C	COX8	1.09	Cytochrome c oxidase
YHR051W	COX6	1.02	Cytochrome c oxidase
YOL077W-A	ATP19	1.58	F1F0 ATP synthase
YLR295C	ATP14	1.37	F1F0 ATP synthase
YPR020W	ATP20	1.28	F1F0 ATP synthase
YPL078C	ATP4	1.04	F1F0 ATP synthase
YMR267W	PPA2	1.47	Mitochondrial inorganic pyrophosphatase
YPL036W	PMA2	1.80	Plasma membrane H ⁺ -ATPase
YFR015C	GSY1	2.03	Glycogen synthase
YLR258W	GSY2	1.22	Glycogen synthase
YJL137C	GLG2	1.13	Glycogenin glucosyltransferase
YKR058W	GLG1	1.27	Glycogenin glucosyltransferase
YPR160W	GPH1	1.59	Glycogen phosphorylase
YMR105C	PGM2	1.05	Phosphoglucomutase
YGR287C	IMA1	1.38	Alpha-1,6-glucosidase
YIL172C	IMA3	1.32	Alpha-glucosidase
YOL157C	IMA2	1.25	Alpha-1,6-glucosidase
YBR018C	GAL7	1.03	Galactose-1-phosphate uridyl transferase
YLR081W	GAL2	2.74	Galactose permease
YJR160C	MPH3	1.62	Alpha-glucoside permease
YJR159W	SOR1	2.54	Sorbitol dehydrogenase
YFL053W	DAK2	2.33	Dihydroxyacetone kinase
YNR073C	MAN2	2.11	Mannitol dehydrogenase
YEL070W	DSF1	1.97	Mannitol dehydrogenase

Glycolysis/Gluconeogenesis

The growth and development of yeast cells, including the synthesis of nucleic acids and proteins, the delivery of biofilms, and transport functions, all require energy consumption. What is interesting is that we found that the overall expression of genes involved in the sugar metabolism pathway is up-regulated (Table 4). *HXK1* and *HXK2* encode hexokinase, which catalyzes glucose-phosphorus to glucose-6-phosphate, The expression level was significantly up-regulated under formic acid stress, indicating that the utilization of glucose by yeast was accelerated. *TDH1* is a gene that regulates glyceraldehyde-3-phosphate dehydrogenase activity, and its upregulation accelerates the conversion of glyceraldehyde-2-phosphate to pyruvic acid. *PGK1* and *GPM1* encode 3-phosphoglycerate kinase and phosphoglycerate mutase, respectively. which are mainly involved in the conversion of 1,3-diphosphoglyceric acid to 2-phosphoglyceric acid in glycolysis, and produce a small amount of ATP. *ENO2* and *ENO1* encode enolase, which play a role in the main metabolic pathways of glycolysis and gluconeogenesis (Kornblatt et al. 2013). *PYK2* encodes pyruvate kinase, which is an important rate-limiting enzyme in the glycolysis pathway, and the of its activity helps to accelerate the smooth progress of EMP (Mitsui et al. 2020). *PDC6*, which regulates the activity of pyruvate kinase, is down-regulated, which slows down the conversion of pyruvate to acetaldehyde. In addition, the expression of alcohol dehydrogenase gene *ADH3* was up-regulated, but the expression of the enzyme gene *ADH2*, which is the reverse reaction of ethanol production of acetaldehyde, was down-regulated. Studies have shown that knocking out *ADH2* gene in *S. cerevisiae* strain As2.4 can increase ethanol production by 52% (Ye et al. 2016). *ALD4*, which is related to aldehyde dehydrogenase, is down-regulated and the conversion of acetaldehyde is reduced. This may be due to the fact that yeast gradually adapts to environmental pressure by regulating its metabolic activity and avoiding the decline of activity, thus affecting the fermentation environment. *PCK1* encodes phosphoenolpyruvate carboxykinase, which catalyzes the conversion of oxalylacetic acid to allylpyruvate. It is a reverse reaction of glycolysis pathway, and the down-regulation of its transcription level promotes the metabolism of EMP pathway.

In yeast, glucose transport is mainly accomplished by the transporter encoded by the HXT gene, a member of the Major facilitator superfamily (MFS) (Özcan et al. 1999). In this study, we found that the expression of glucose transporters encoded by *HXT1*, *HXT2*, *HXT3*, *HXT4*, *HXT6*, *HXT7* and *HXT15* were significantly up-regulated (Table 4). *HXT1* and *HXT3* encode low affinity glucose transporters, *HXT2*, *HXT4*, *HXT6* and *HXT7* all encoded glucose transporters with high affinity. Among them, *HXT1* was induced under high glucose concentration, while the expressions of *HXT6* and *HXT7* were inhibited under high glucose concentration. *HXT2* and *HXT4* were induced under low glucose concentration (Özcan et al. 1996). In addition, *SNF3* does not directly encode sugar transporters, but acts as an extracellular plasma membrane glucose sensor, and at the same time assists in the induction of expression of *HXT1* and *HXT3* (Özcan et al. 1999). It is suggested that yeast cells respond to formic acid stress mainly by regulating the expression of glucose transporters, thus improving the tolerance of formic acid.

Table 4 Differentially expressed genes related to glycolysis/gluconeogenesis pathway under formic acid stress

Gene ID	Gene Name	Log2 (fold change)	Description
YFR053C	HXK1	2.07	Hexokinase isoenzyme
YGL253W	HXK2	1.15	Hexokinase isoenzyme
YHR174W	ENO2	1.73	Enolase II
YGR254W	ENO1	1.16	Enolase I
YOR393W	ERR1	1.72	Putative phosphopyruvate hydratase
YMR323W	ERR3	1.18	Enolase
YJL052W	TDH1	1.21	Glyceraldehyde-3-phosphate dehydrogenase
YOR347C	PYK2	1.21	Pyruvate kinase
YMR083W	ADH3	1.21	Mitochondrial alcohol dehydrogenase isozyme III
YKL152C	GPM1	1.06	Tetrameric phosphoglycerate mutase
YCR012W	PGK1	1.00	3-phosphoglycerate kinase
YGR087C	PDC6	-1.06	Minor isoform of pyruvate decarboxylase
YKR097W	PCK1	-1.13	Phosphoenolpyruvate carboxykinase
YMR303C	ADH2	-1.53	Glucose-repressible alcohol dehydrogenase II
YOR374W	ALD4	-1.46	Mitochondrial aldehyde dehydrogenase
YDR345C	HXT3	4.84	Low affinity glucose transporter
YHR094C	HXT1	3.49	Low-affinity glucose transporter
YDR343C	HXT6	3.47	High-affinity glucose transporter
YHR092C	HXT4	2.99	High-affinity glucose transporter
YDL245C	HXT15	2.93	Putative transmembrane polyol transporter
YDR342C	HXT7	2.87	High-affinity glucose transporte
YMR011W	HXT2	1.88	High-affinity glucose transporter
YDL194W	SNF3	1.63	Plasma membrane low glucose sensor

Meiosis and cell cycle

As a special way of cell proliferation, meiosis also has a certain impact on cell proliferation. Compared with the control group, the expression of genes involved in meiosis in formic acid-treated yeast cells was all up-regulated (Table 5). *RIM15* is responsible for activating the activity of meiotic genes. *RAD53*, *DDC1* and *MRC1* control the S-phase and G1/G2 DNA damage checkpoints. *SWM1* and *APC2* are essential genes that promote the activity of the complex/loop body (APC/C) in late meiosis. *MCM7*, *MCM4*, and

MCM2 encode proteins for DNA replication, which are part of the *MCM2-7* hexamer helicase complex, participate in the starting point of DNA replication in G1, and can promote the melting and extension of S-phase DNA. *ESP1* is an isolating enzyme that cuts the meiosis-adhesin subunit Rec8p along the chromosome arm during meiosis I and the centromeric site during meiosis II, and its expression is inhibited by Pds1p. The subunit synthesis of lectin complex (encoded by *SMC4*) increased, and the lectin recombines chromosomes during mitosis and meiosis. Therefore, yeast cells can respond to formic acid stress through sexual reproduction, because sexual reproduction can improve the adaptability of offspring to the environment through gene recombination.

Cyclin is a type of protein that expresses, accumulates, and decomposes in a cell cycle-specific or phased manner, and they together with cyclin-dependent kinases affect the operation of cell cycle. The cell cycle is mainly divided into the early stage of DNA synthesis (G1 stage), the DNA synthesis stage (S stage), the late DNA synthesis stage (G2 stage) and the cell division stage (M stage). Most cell cycle-related genes are up-regulated (Table 5), such as *CLB1*, *CLB2*, *CLB6*, *CLN1*, *CLN2*, *CLN3*, *SPS4*, *PCL1*, *DUN1*, *MBP1*. *PCL1* encodes the G1/S phase-specific cyclic protein involved in cell cycle regulation, interacts with the cyclin-dependent kinase Pho85p, and participates in the regulation of polarized growth and morphogenesis and progression during cell growth. Its up-regulated expression means Promote the transition from G1 phase to S phase. The up-regulated expression of cyclin *CLN1*, *CLN2* and *CLN3* genes means that they activate *Cdc28p* kinase to promote the transition from G1 to S phase. *MBP1* is involved in the regulation of cell cycle from G1 to S phase. In addition, *CLB6* can activate *Cdc28p* to promote the initiation of DNA synthesis, and plays a role in the formation of mitotic spindles together with *Clb3p* and *Clb4p*. *CLB1* and *CLB2* (cyclin) can activate *Cdc28p* to promote the transformation from G2 to M phase (Hadwiger et al. 1989; Wittenberg et al. 1990). *CLB1* is involved in meiosis, but *CLB2* is only involved in mitosis (Grandin et al. 1993). It is worth noting that the up-regulated expression of *DUN1*, as a cell cycle checkpoint protein gene, and *Dun1p*, as a signal transducer for cell cycle arrest and transcription response to damaged or unreplicated DNA, Its up-regulated expression often means DNA damage. In addition, the gene *SPS4* regulating spore formation was up-regulated, and the yeast showed meiosis under nitrogen stress, forming four haploid ascospores (Hill et al. 2007). Studies have shown that meiosis, spore formation and pseudohypha growth are the responses of *S. cerevisiae* to nitrogen starvation (Schrder et al. 2000). Therefore, we believe that formic acid stress may cause nitrogen starvation, and yeast cells can improve the adaptability of offspring to the environment through sexual reproduction and spore formation, thereby improving the tolerance of formic acid.

Table 5 Differentially expressed genes related to meiosis and cell cycle under formic acid stress

Gene ID	Gene Name	Log2 (fold change)	Description
YDR260C	SWM1	2.13	Subunit of the anaphase-promoting complex
YPL153C	RAD53	1.85	DNA damage response kinase
YBR202W	MCM7	1.71	The Mcm2-7 hexameric helicase
YCL061C	MRC1	1.59	S-phase checkpoint protein
YLR086W	SMC4	1.35	Subunit of the condensin complex
YPR019W	MCM4	1.34	Heterohexameric MCM2-7 complexes
YLR127C	APC2	1.15	Subunit of the Anaphase-Promoting Complex
YGR098C	ESP1	1.14	Separase/separin
YPL194W	DDC1	1.07	DNA damage checkpoint protein
YBL023C	MCM2	1.03	Protein involved in DNA replication
YFL033C	RIM15	1.02	Protein kinase
YGR108W	CLB1	2.70	B-type cyclin
YOR313C	SPS4	2.55	Participate in sporulation
YNL289W	PCL1	2.44	Cyclin
YPR119W	CLB2	1.74	B-type cyclin
YDL101C	DUN1	1.19	Cell-cycle checkpoint S/T protein kinase
YMR199W	CLN1	1.16	G1 cyclin involved in regulation of the cell cycle
YDL056W	MBP1	1.12	Transcription factor
YGR109C	CLB6	1.11	B-type cyclin
YPL256C	CLN2	1.05	G1 cyclin involved in regulation of the cell cycle
YAL040C	CLN3	1.015	G1 cyclin involved in cell cycle progression

MAPK signaling pathway

Through KEGG enrichment analysis, it was found that formic acid could significantly change the MAPK signaling pathway in cells, that is, under formic acid stress, cells were controlled to make corresponding environmental response by activating intracellular signal transduction pathways. The most important signal transduction pathway in yeast cells is mitogen-activated protein kinase (MAPK) pathway (Levin et al. 2005). At least four MAPK cascades reactions in *S.cerevisiae* cells are composed of yeast responses to different physiological stimuli: cell wall integrity pathway, pheromone response pathway, high osmotic pressure glycerol pathway and filamentous or invasive growth pathway (Chen et al. 2007). In our study, the expressions of *MF(ALPHA)1*, *MF(ALPHA)2*, *STE3*, *STE2*, and *SST2* involved in the pheromone

signaling pathway were all up-regulated (Table 6). *MF(ALPHA)1* and *MF(ALPHA)2*, as mating pheromone α factors, are composed of a cells and interact with mating type a cells, inducing cell cycle arrest and other mating reactions. *STE3* and *STE2*, as receptors of α factor pheromone, cascade with MAP kinase, and are required for transcription in a cells and mating with a cells. The ligand-bound receptor is endocytosed and recycled to the plasma membrane. As GTPase activator protein of *Gpa1p*, *SST2* can regulate the desensitization of α -factor pheromone, and it also necessary to prevent receptor-independent signal transduction in mating pathway. *Bnilp* is the key to the formation of linear actin filaments and participates in the cell processes that need to polarize actin clusters, such as budding and mitotic spindle orientation (Matheos et al. 2004; Yu et al. 2008). *Rholp* can also activate Bnilp, and Rom1p and Rom2p as the guanine nucleotide exchange factors of Rho1p and Rho2p can activate Rholp (Ozaki et al. 1996; Krause et al. 2012). *TUS1* can regulate Rho1p activity and participate in the interaction of cell wall integrity signaling pathway with Rgl1p. *SSK2* encodes the MAP kinase of the mitogen-activated signaling pathway, and interacts with Ssk1p to cause autophosphorylation and activation of Ssk2p, thereby phosphorylation of Pbs2p, and also mediates the cytoskeleton that actin recovers from osmotic stress. As a stress-induced dual-specific MAP kinase phosphatase, *SDP1* negatively regulates Slt2p MAP kinase through direct dephosphorylation. *SLN1*, as a transmembrane histidine phosphate transfer kinase and osmotic sensor, regulates the cascade of MAP kinases and has a transmembrane protein with an intracellular kinase domain that signals to Ypd1p and Ssk1p. *FLO11* is a gene regulating cell filamentous growth. *FKS3* is involved in the assembly of spore wall proteins required for flocculation and biofilm formation. The up-regulation of these genes indicates that formic acid has adverse effects on the growth of *S. cerevisiae*. The tolerance of *S. cerevisiae* to formic acid may be improved by regulating the signal transduction pathway and filamentous or invasive growth pathway of pheromone.

Table 6 Differentially expressed genes related to MAPK signaling pathway under formic acid stress

Gene ID	Gene Name	Log2 (fold change)	Description
YLR452C	SST2	2.13	GTPase-activating protein for Gpa1p
YPL187W	MF(ALPHA)1	2.04	Mating pheromone alpha-factor
YIR019C	MF(ALPHA)2	1.63	Mating pheromone alpha-factor
YLR425W	TUS1	2.01	Guanine nucleotide exchange factor (GEF)
YIL113W	SDP1	1.88	MAP kinase phosphatase
YKL178C	STE3	1.87	Receptor for a factor pheromone
ROM1	ROM1	1.707	Guanine nucleotide exchange factor (GEF)
YGL089C	FLO11	1.66	Pseudohyphal and invasive growth
YLR371W	ROM2	1.47	Guanine nucleotide exchange factor (GEF)
YNL271C	BNI1	1.46	Nucleates the formation of linear actin filaments
YMR306W	FKS3	1.45	Protein involved in spore wall assembly
YNR031C	SSK2	1.31	MAP kinase
YIL147C	SLN1	1.23	Transmembrane histidine phosphotransfer kinase
YFL026W	STE2	1.20	Receptor for a factor pheromone

Amino acid metabolism

Amino acids are the main metabolites of cells. In the process of cell metabolism, amino acids can not only participate in cell construction as important precursor substances, but also participate in intracellular biochemical reactions and metabolic regulation by forming catalytic enzymes. Transcriptome analysis revealed that the differentially expressed genes related to the amino acid metabolism pathway also showed periodic changes (Table 7). The most significant ones are involved in methionine and methionine synthesis pathways (*MET17*, *MET6*, *MET3*, *STR3*, *CYS4*, *CYS3*, *SAM2*, *SAH1*, *SPE3*, *SPE2*, *MDE1*) and valine. The differentially expressed genes of leucine and isoleucine biosynthesis (*ILV2*, *ILV3*, *ILV5*, *POT1*, and *Leu9*) were significantly down-regulated. Van et al. (2011) studied the response of *Lactobacillus plantarum* WCFS1 to the down-regulation of amino acid metabolism-related genes after 8% ethanol treatment to support this. In *S. cerevisiae* cells, the amino acid metabolism pathway is reduced, which may reduce energy requirements and enhance the viability of yeast cells. Weber et al. (2005) found that the differential genes related to cell growth and division and protein synthesis are significantly inhibited under external environmental stress, which was precisely to reduce energy loss and achieve self-protection. It can be seen that the reduction of amino acid metabolism in yeast cells may be to reduce energy requirements and rationally allocate the energy produced by themselves to make them survive the stress of formic acid.

Studies have shown that yeasts are faced with multiple environmental pressures such as high ethanol, high acid and insufficient nutrients, and nitrogen source is the most common factor limiting the growth of yeasts (Bely et al. 1990). *SER3* encodes 3-phosphoglycerate dehydrogenase, which catalyzes the synthesis of serine and glycine. Up-regulation of its expression accelerates the synthesis of these two amino acids. *CHA1* encodes L-serine deaminase, which catalyzes the degradation of L-serine and threonine. requiring the use of serine or threonine as the only nitrogen source, indicating that maintaining the balance of carbon and nitrogen metabolism in yeast cells plays an important role under formic acid stress. Glutathione is an important antioxidant substance, which can reduce active oxygen free radicals to generate oxidized glutathione to exert its antioxidant function and protect important organelles in the cell from damage (Mailloux et al. 2013). In this study, it was found that genes involved in the glutathione metabolism pathway were up-regulated (Table 7). *GSH2* encodes glutathione synthetase, which catalyzes the synthesis of glutathione from γ -glutamylcysteine and glycine. *GPX2* encodes glutathione peroxidase, which protects cells from phospholipid hydroperoxides and non-phospholipid peroxides during oxidative stress (Izawa et al. 1995; Grant et al. 1998). Since glutathione is responsible for the redox state in cells and acts as a protective agent against reactive oxygen species (Morano et al. 2012), these results indicate that formic acid stress can disturb the redox balance of yeast. *GND1* and *GND2* encode glucose-6-phosphate dehydrogenase, which catalyzes the production of ribulose-5-phosphate from phosphogluconate, produces a large amount of NADPH in the pentose phosphate pathway, provides reducing power for various synthesis reactions of cells, and maintains The level of redox in the cell. At the same time provide synthetic DNA, RNA, tryptophan, tyrosine and other biological synthesis of the premise substances 4-phosphoerythritol and 5-phosphoribose. It is because of the increased activity of glucose 6-phosphate dehydrogenase that yeast cells have more raw materials to synthesize cells, thus improving the tolerance of GGSF16 to formic acid.

Table 7 Differentially expressed genes related to amino acid metabolism pathway under formic acid stress

Gene ID	Gene Name	Log2 (fold change)	Description
YER091C	MET6	-1.08	Methionine synthase
YAL012W	CYS3	-1.27	Cystathionine gamma-lyase
YER043C	SAH1	-1.29	S-adenosyl-L-homocysteine hydrolase
YGL184C	STR3	-1.33	Peroxisomal cystathionine beta-lyase
YOL052C	SPE2	-1.34	S-adenosylmethionine decarboxylase
YJR024C	MDE1	-1.34	5'-methylthioribulose-1-phosphate dehydratase
YLR303W	MET17	-1.54	O-acetyl homoserine-O-acetyl serine sulfhydrylase
YGR155W	CYS4	-1.62	Cystathionine beta-synthase
YPR069C	SPE3	-1.78	Spermidine synthase
YJR010W	MET3	-2.06	ATP sulfurylase
YDR502C	SAM2	-2.54	S-adenosylmethionine synthetase
YMR108W	ILV2	-2.14	Acetolactate synthase
YJR016C	ILV3	-2.11	Dihydroxyacid dehydratase
YLR355C	ILV5	-1.41	Acetohydroxyacid reductoisomerase
YIL160C	POT1	-1.31	3-ketoacyl-CoA thiolase
YOR108W	LEU9	-1.12	Alpha-isopropylmalate synthase II
YIL074C	SER3	2.09	3-phosphoglycerate dehydrogenase
YCL064C	CHA1	1.07	Catabolic L-serine (L-threonine) deaminase
YGR256W	GND2	1.76	6-phosphogluconate dehydrogenase
YHR183W	GND1	1.64	6-phosphogluconate dehydrogenase
YBR244W	GPX2	1.56	Phospholipid hydroperoxide glutathione peroxidase
YOL049W	GSH2	1.01	Glutathione synthetase

Conclusions

In this study, transcriptomics was used to study the cytotoxicity of formic acid to *S. cerevisiae* GGSF16 and its molecular mechanism. Formic acid can obviously inhibit the growth of yeast cells, and cause cell damage and even death by changing the structure of proteins and carbohydrates on the cell wall and cell membrane. Notably, transcriptomics data show that formic acid stress can not only inhibit protein biosynthesis, but also induce oxidative stress, resulting in autophagy, impaired intracellular ATP

production and increased consumption, leading to impaired cellular physiological and metabolic functions. GGSF16 provides sufficient energy to resist formic acid stress by accelerating energy metabolism, enhancing electron transfer and ATP synthesis. It can also reduce the expression of some genes related to the biosynthesis of energy-consuming metabolic pathways such as intracellular amino acids, thereby achieving an energy-saving strategy, and It can rationally use its own energy to synthesize substances needed to cope with stress to protect cells. In addition, sexual reproduction and spore formation can improve the adaptability of offspring to the environment, promote the absorption of nutrients, and improve the tolerance of cells to formic acid. This review preliminarily revealed the molecular response mechanism of *S. cerevisiae* under formic acid stress, which provided a theoretical basis for the subsequent study on the tolerance of cell inhibitors in lignocellulose hydrolysates.

Declarations

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Conflict of interest The authors declare that they have no conflict of interest.

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Figures

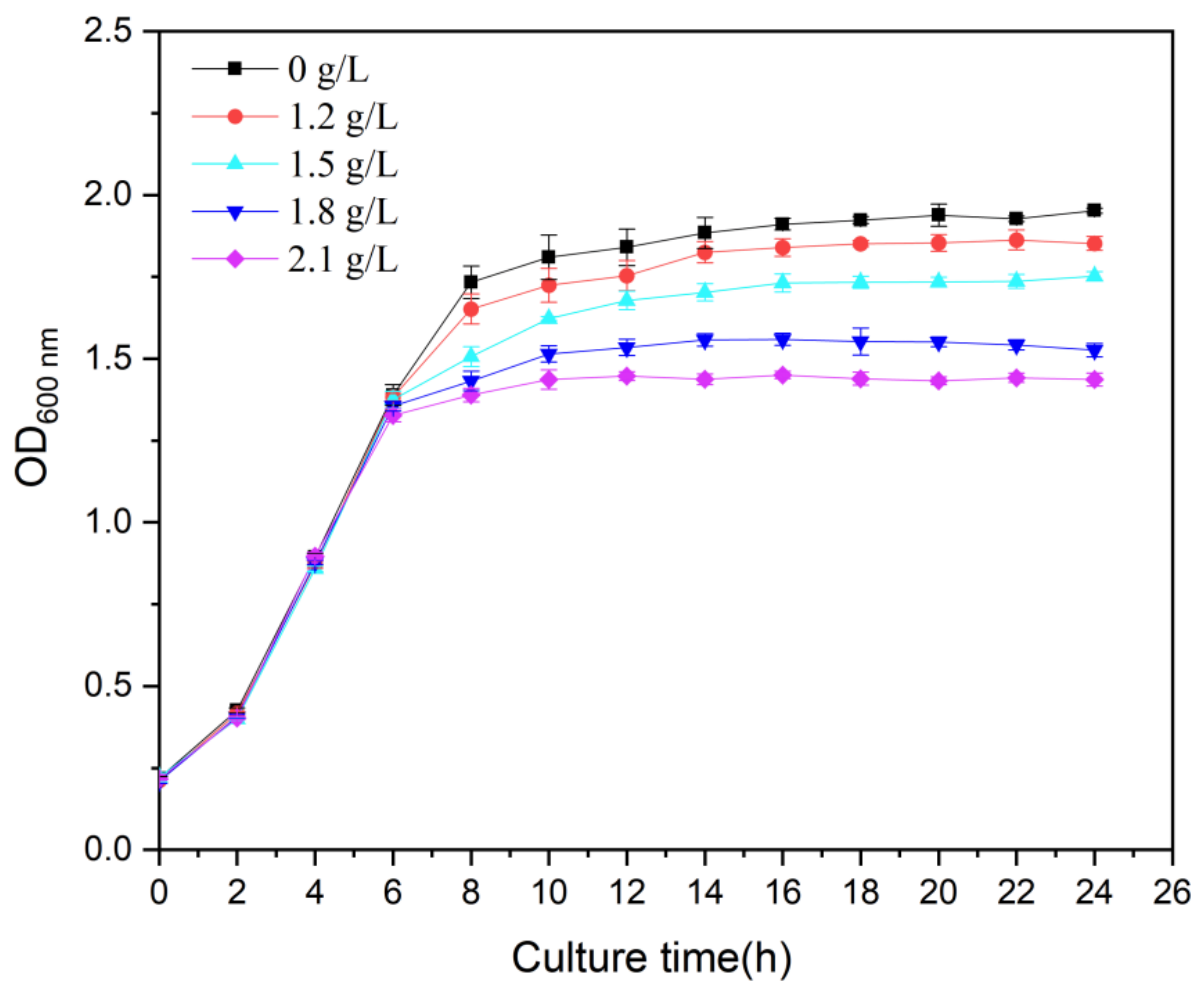


Figure 1

The effect of formic acid on the growth of yeast cells

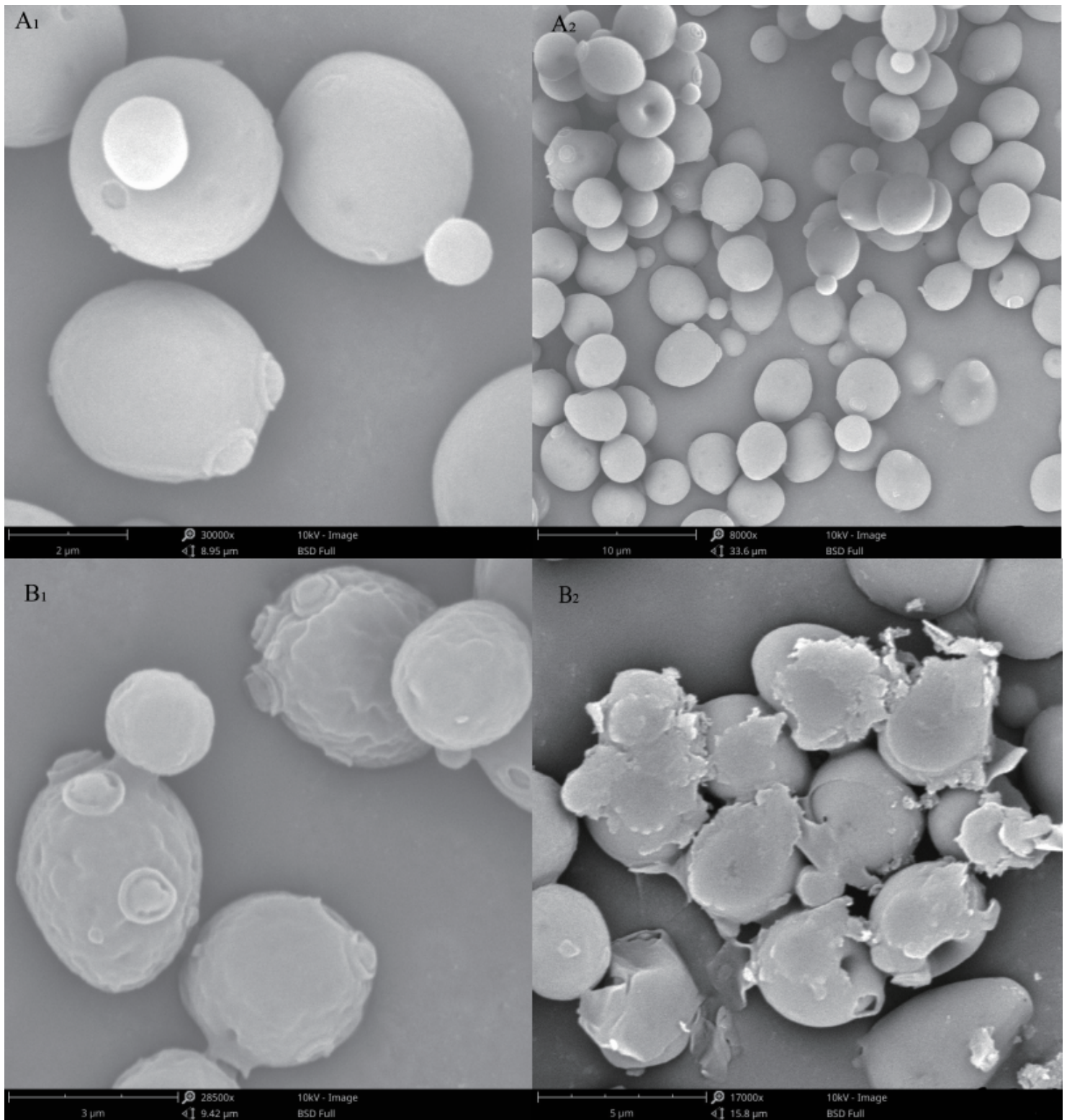


Figure 2

Scanning electron microscope of the GGSF16 (A)Control group; (B)Treatment group

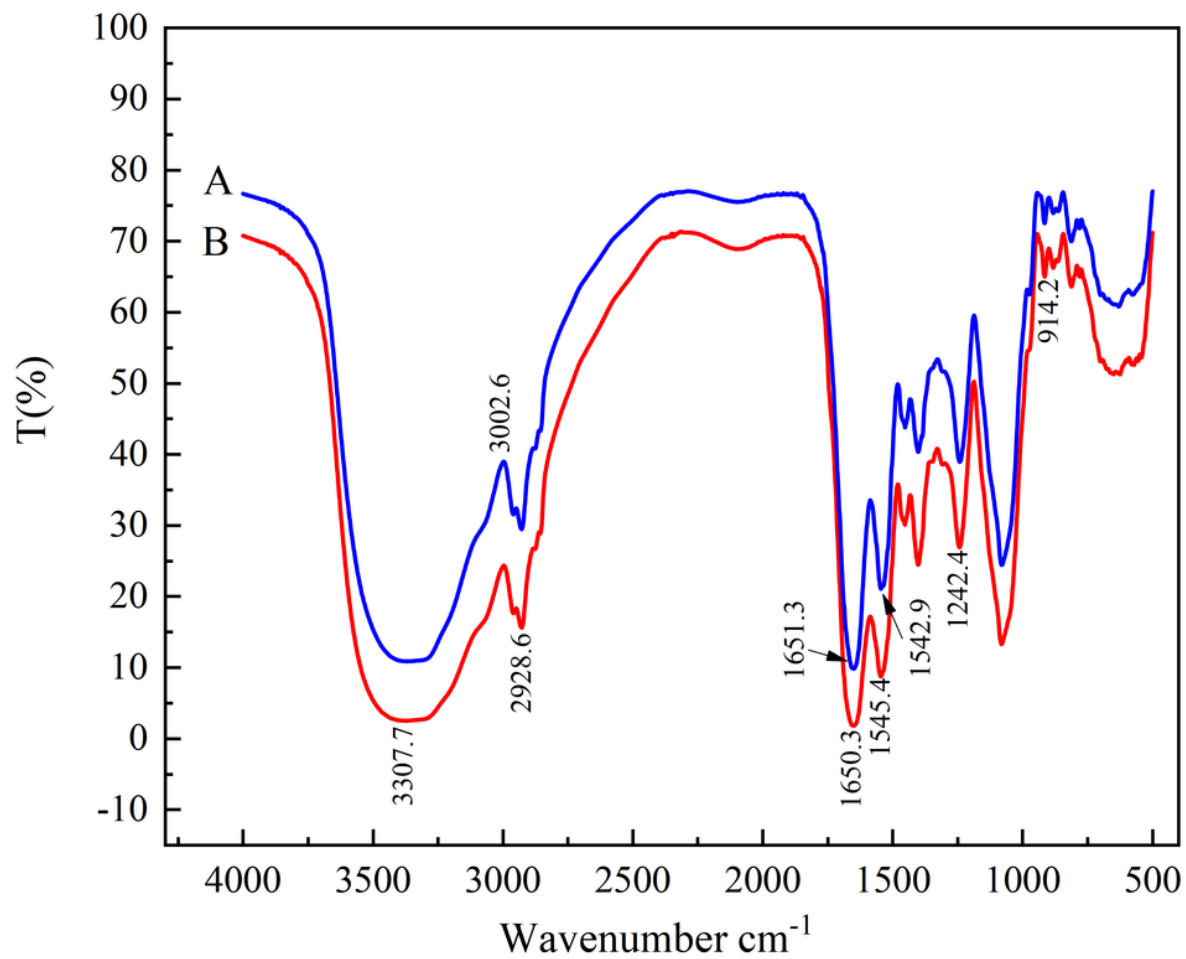


Figure 3

Infrared spectra of formic acid before and after treatment (A) Treatment group (B) Control group

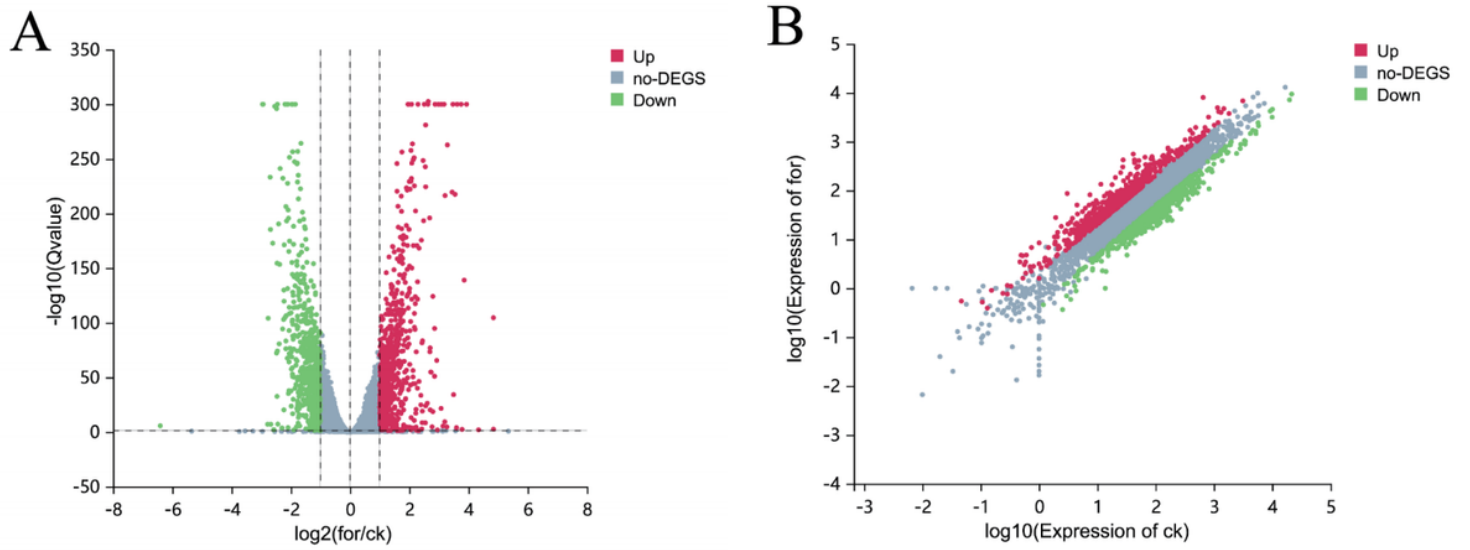


Figure 4

DEGs between control group and formic acid treatment group

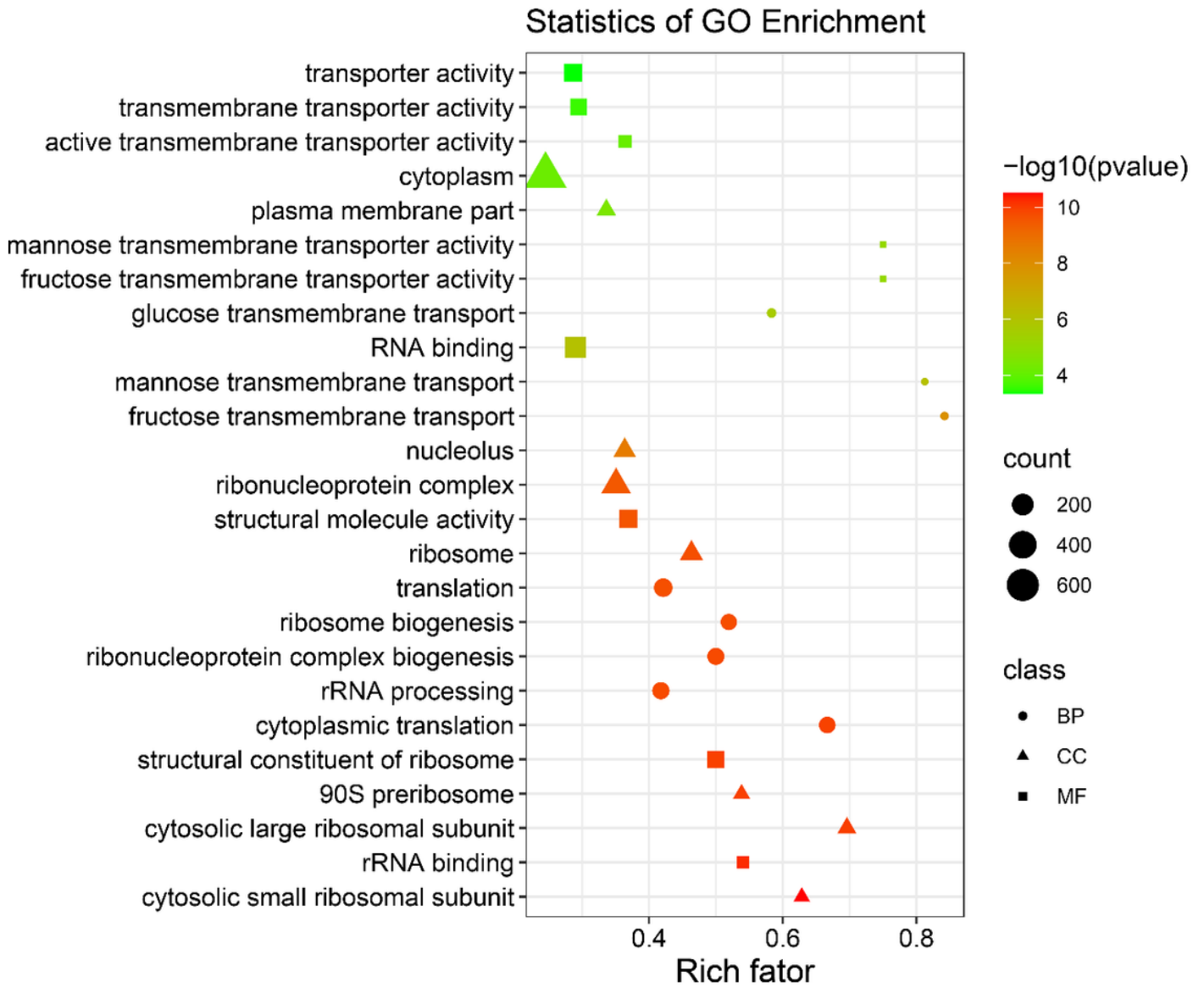


Figure 5

GO enrichment analysis of the DEGs

Statistics of KEGG Enrichment

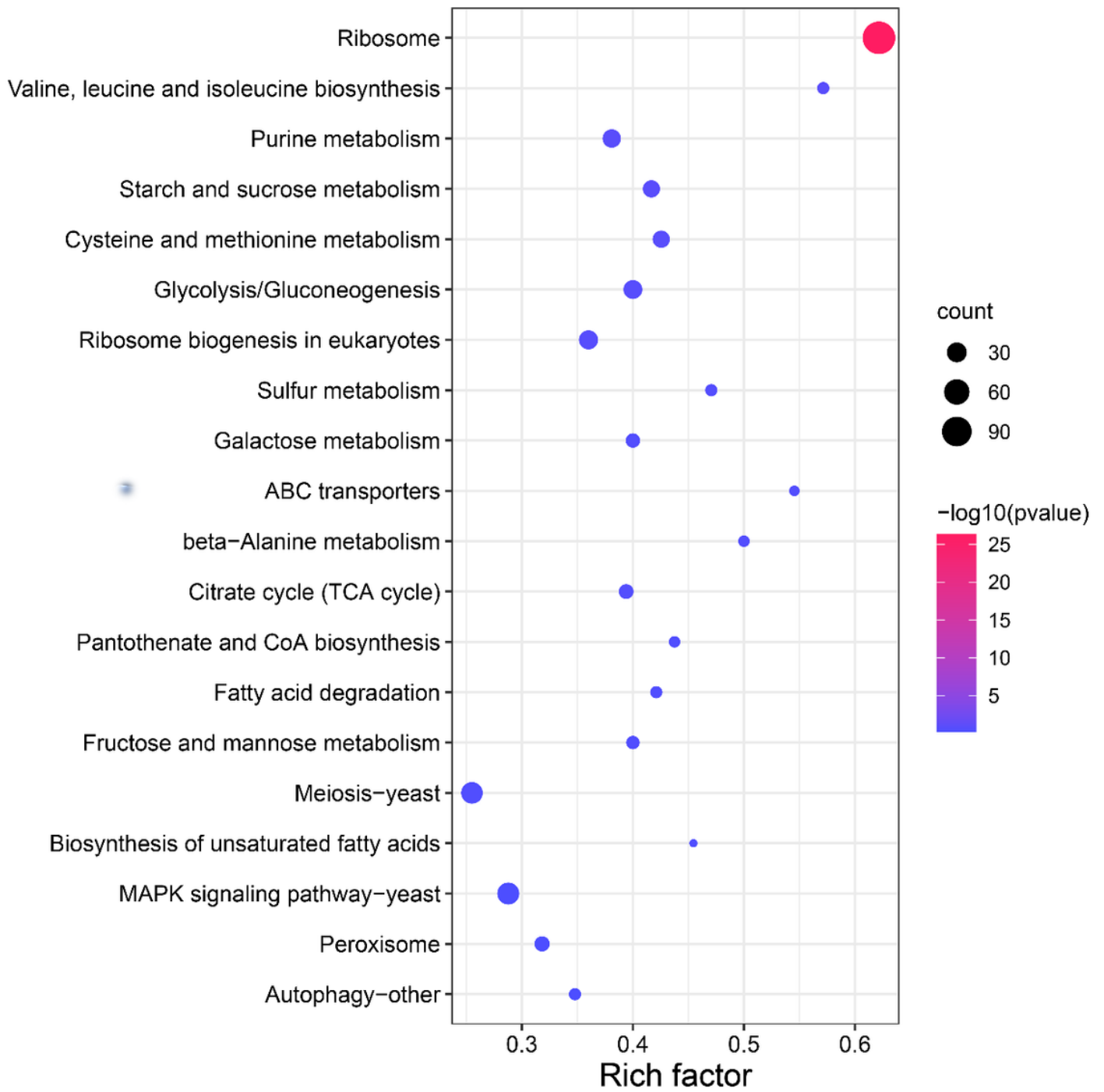


Figure 6

KEGG enrichment analysis of the DEGs

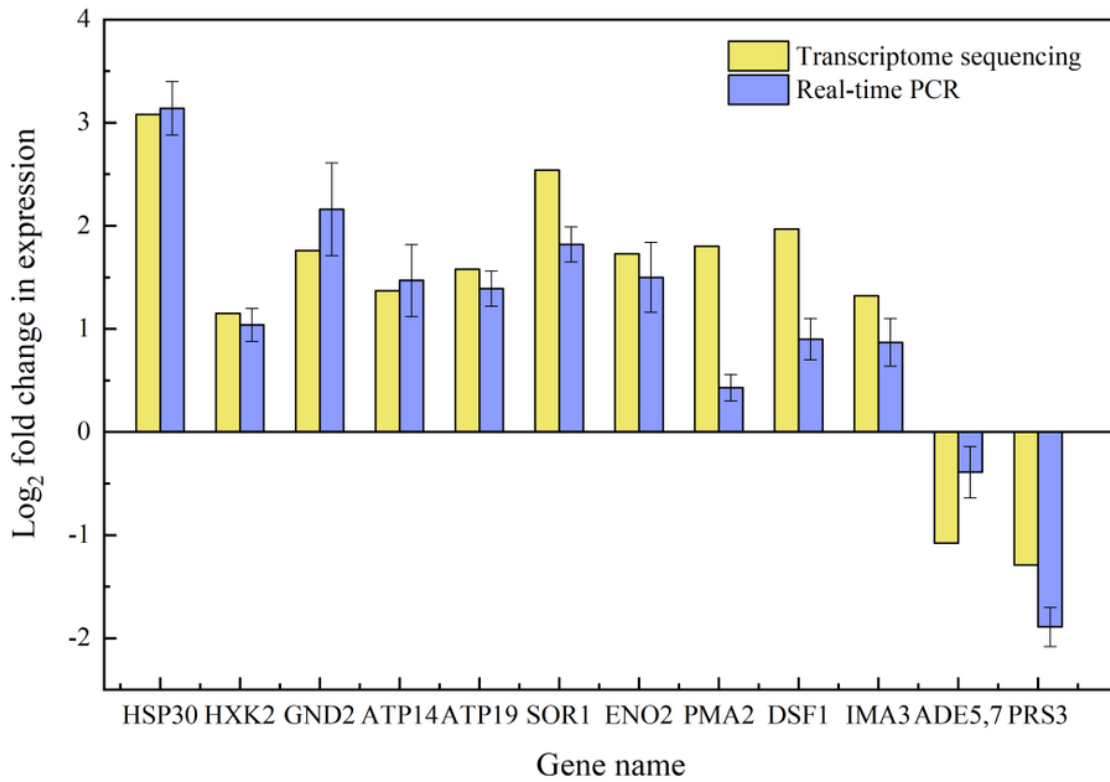


Figure 7

Fluorescence quantitative PCR verification