



# RNA-seq transcriptomic analysis of green tea polyphenols regulation of differently expressed genes in *Saccharomyces cerevisiae* under ethanol stress

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## Abstract

*Saccharomyces cerevisiae* has been widely used to produce alcoholic beverages and bio-fuels; however, its performance is remarkably compromised by the increased ethanol concentration during the fermentation process. In this study, RNA-sequence analysis was used to investigate the protective effect of green tea polyphenols (GTP) on *S. cerevisiae* cells from ethanol-induced damage. GO and KEGG analysis showed that to deal with the stress of ethanol, large amounts of genes related to cell wall, cell membrane, basic metabolism and redox regulation were significantly differentially expressed ( $P < 0.05$ ), while these undesired changes could be partly relieved by administration of GTP, suggesting its potential to enhance the ethanol tolerance of *S. cerevisiae*. The present study provided a global view of the transcriptomic changes of *S. cerevisiae* in response to the accumulation of ethanol and the treatment of GTP, which might deepen our understanding about *S. cerevisiae* and the fermentation process, and thus benefit the development of the bioethanol production industry.

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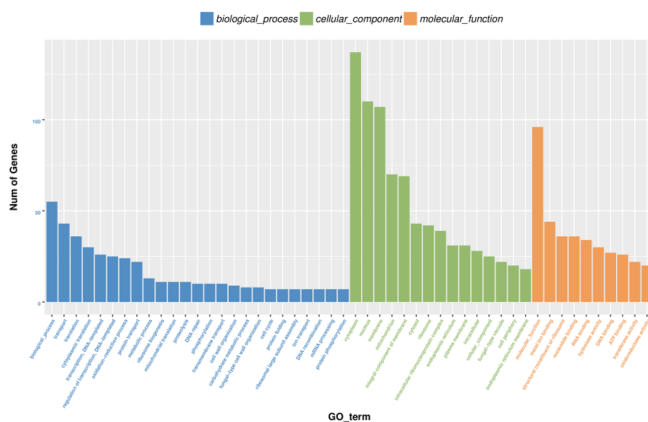
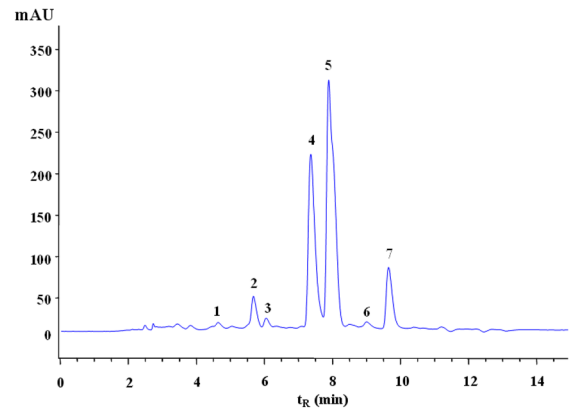
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## Graphical abstract

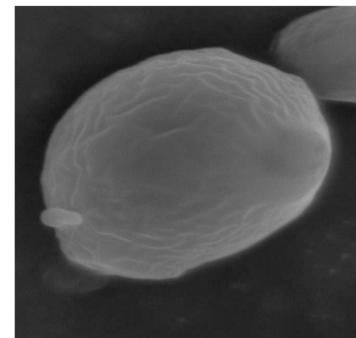


Green tea

Extraction  
Purification



RNA-seq

*Saccharomyces cerevisiae* under ethanol stress

**Keywords** Green tea polyphenols · *Saccharomyces cerevisiae* · Ethanol stress · Transcriptome

## Introduction

The yeast *Saccharomyces cerevisiae* with excellent potential in ethanol producing, has traditionally and extensively served as dominant species for the fermentation of alcoholic beverages, such as wine, beer and sake, for thousands of years (Gallone et al. 2016). In addition, *S. cerevisiae* has made a significant contribution to the growing ethanol biofuel production which effectively relieves the urgent energy crisis and environmental concerns (Rodionova et al. 2017). Compared with other microorganisms, *S. cerevisiae* exhibits higher ethanol yield and tolerance, however, its successful performance during bioethanol production processes is still compromised by several types of environmental stresses, including increasing ethanol concentration, high osmotic pressure, oxidation and so forth. Among these stress factors, high level of ethanol in the culture broth has been regarded as the major one that induces oxidative stress, affects

cell growth and vitality, inhibits the activity of some key enzymes, and interferes with various cell metabolisms, leading to the reduction of fermentation productivity and ethanol yield (Kasavi et al. 2016). It is known that a cost-effective bioethanol fermentation process is dependent on a rapid and high yielding-conversion of carbohydrate to ethanol, which can be realized by the improvement of the survival and performance of *S. cerevisiae* cells under industrial conditions (Laluce et al. 2012).

In China, tea has been used to treat alcoholic intoxication for more than one millennium (Zhang et al. 2014), and considerable reports have supported the protective action of tea polyphenol against ethanol-induced toxicity. Augustyniak et al. (2005) pointed out green tea was able to exert beneficial effect to rats intoxicated with ethanol through reducing oxidative stress due to its high antioxidant activity. Green tea, as a 'non-fermented' tea, is mainly processed from leaves and buds of *Camellia*

*sinensis* var. *sinensis* and has been consumed around the world. Chemical constituent studies revealed that green tea contained abundant polyphenolic compounds which were believed to be the effective components contributing to the detoxication of ethanol (Zhang et al. 2014). Among these compounds, catechins (flavan-3-ols) were the predominant ones and found to be higher in green tea than in others, such as black tea, oolong tea, and Pu-Erh tea (Lin et al. 2003). Considering its wide range of positive bioactivities, green tea polyphenol (GTP) has become a hot study topic in food, biology and chemistry fields nowadays. However, to the best of our knowledge, no studies have been carried out to investigate the potential application of GTP to ameliorating the ethanol stress and ethanol-induced damage in *S. cerevisiae* during ethanol production.

RNA sequencing (RNA-Seq), as a revolutionary tool for transcriptomics profiling through deep-sequencing technologies, has already been successfully applied to *S. cerevisiae* (Nagalakshmi et al. 2008), which can improve our view of the complexity of eukaryotic transcriptomes. Various genes regulate the phenotypic characteristics of *S. cerevisiae*, and the expression of different genes involved in multiple biological functions and processes were found to be remarkably altered in response to ethanol stress (Li et al. 2017). Therefore, GTP as one of the most popular natural antioxidants in food industry attracted our attention. In this study, RNA-seq was used to investigate the influence of GTP on the gene expression profiles of *S. cerevisiae* 131 (Sc131) under ethanol stress. The present study deepened our understanding of the molecular response to ethanol in *S. cerevisiae* and suggested that GTP should be a promising functional additive for the industrial ethanol fermentation.

## Materials and methods

### Chemicals and reagents

Polyamide resin was obtained from Qingdao Ocean Chemical Co., Ltd. (Qingdao, China). Standards of (-)-epigallocatechin (EGC) (> 98%), (-)-epigallocatechin gallate (EGCG) (> 98%) and (-)-epicatechin gallate (ECG) (> 98%) were purchased from Funakoshi (Tokyo, Japan). Standard of theophylline (> 98%) was from Sigma-Aldrich (St. Louis, MO, USA). Standards of (+)-catechin (C) and (-)-gallocatechin gallate (GCG) were prepared according to the reported methods with minor modifications (Zhang et al. 2015). HPLC grade methanol was obtained from Jiangsu Hanbon Science and Technology Co., Ltd. (Jiangsu, China). All other chemicals and reagents were analytical grade commercial products.

### Preparation of GTP

Green tea was obtained locally (Ningbo, Zhejiang province) and GTP was prepared according to the reported method with some modifications (Zhang et al. 2014). Briefly, 1000 g of tea powder was extracted with 16 L of distilled water at 96 °C for 40 min. Upon extraction, the extract was centrifuged at 4500×g for 15 min, and the resulting insoluble residue was treated again as described above. The supernatants were combined and concentrated, and the resulting residue was dissolved, filtered, and loaded onto a polyamide column. The column was eluted with 80% ethanol, and the elution was monitored by measuring the absorbance at 280 nm and auto-collected. The eluted fractions were analyzed by high-performance liquid chromatography (HPLC), and the desired fractions were collected, concentrated, loaded onto the polyamide column, and treated again as described above. As a result, the fractions containing GTP were concentrated and lyophilized by a freeze-dry system.

### HPLC analysis of GTP

The contents of tea catechins were determined according to our reported method (Zhang et al. 2014) using an Agilent 1100 series HPLC (Agilent, CA, USA). The separation was achieved on a TSKgel ODS-100Z column (4.6×150 mm, 5 µm, Tosoh, Tokyo, Japan). The mobile phase consisted of formic acid solution (pH 2.5, A) and methanol (B). Elution was performed with a linear gradient as follows: 0-15 min, A from 82 to 40%. The temperature of the column oven was set at 40 °C, the flow rate was set at 1.0 mL/min, and the injection volume was 20 µL.

### Culture conditions of Sc131

The strain Sc131 was given as a present from the Laboratory of Food Microbiology, Wageningen University, Netherlands, which was originally obtained from *masau* (*Ziziphus mauritiana*) fruits. The strain is indigenous and now preserved in food biotechnology laboratory of Ningbo University. Some characters of this strain Sc131 such as tolerance to alcohol and phenotypic tests have been published in our previous study (Li et al. 2017). In addition, we have investigated the interaction between *Saccharomyces cerevisiae* and *Pichia fabianii* in a mixed culture (Bai et al. 2017). Cultures were maintained on yeast extract agar slopes at 4 °C until required. The strain was maintained on YPD plates (1% yeast extract, 2% peptone, 2% glucose, 2% agar). YPD medium (1% yeast extract, 2% peptone, 2% glucose) was used for pre-cultivation at 30 °C with aeration and agitation (150 rpm). The activated Sc131 cells were inoculated in 50 mL of fresh YPD medium at 30 °C with an initial OD<sub>600</sub> of 0.1.

Then 0.5 mL of pre-cultured Sc131 was inoculated in 50 mL of high-sugar YPD medium (1% yeast extract, 2% peptone and 10% glucose) and incubated at 30 °C in an orbital shaker at 150 rpm till the mid-exponential phase. Ethanol was added into the experimental cultures to a final ethanol concentration of 10% (v/v) at exponential phase after cultivated for 8 h. Then they were randomly divided into three groups: the control group (control), the ethanol group (ethanol), and the ethanol with GTP group (ethanol-GTP). GTP was added to a final concentration of 0.3% (v/v), based on our previous experiment for the effective ameliorate effect of GTP on the ethanol stress for Sc131 (Fig. S1). The Sc131 cells were cultured in an orbital shaker and monitored by measuring the optical density of the culture medium at 600 nm ( $OD_{600}$ ) with a spectrophotometer. Cells for RNA-seq analysis were collected 8 h after the addition of ethanol. All samples were stored at  $-80$  °C prior to use.

### RNA preparation, library construction and sequencing

Samples containing yeast cells were centrifuged at  $5000\times g$  at 4 °C for 5 min (Li et al. 2017). 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 hot phenol method (Köhler and Domdey 1991). After extraction, mRNAs were enriched using oligo (dT) magnetic beads and broken into short pieces in fragmentation buffer. The first cDNA strand was synthesized by using hexamers as the template, then the second strand cDNA synthesis was subsequently performed using DNA polymerase I and RNase H. Ampure XP beads purified double stranded products using T4 DNA polymerase and Klenow DNA polymerase activity to restore the sticky end of DNA to the flat end. The end of 3' was added with base A and inserted into the linker, and an Ampure XP bead was used for fragment selection. Finally, PCR amplification was used to obtain the final sequencing library. The library was tested and sequenced by Illumina HiSeq 4000. The sequence was read as double ended  $2 \times 150$  bp (P150).

### Reads mapping, annotation and analysis

Using the Illumina paired-end RNA-seq approach, we sequenced the transcriptome, generating a total of million paired-end reads of bp length. This yielded gigabases (Gb) of sequence. Prior to assembly, the low-quality reads were removed including: first, reads containing sequencing adaptors; second, reads containing sequencing primer; third, nucleotides with q quality score lower than 20. Genes whose ratio changed > twofold with  $q < 0.05$  were determined to have significantly different gene expression. The ratios were reported as the up- or down-fold change. Gene descriptions

and annotations were found in the Genome Database of *S. cerevisiae* (<https://www.ncbi.nlm.nih.gov/genome>).

Annotations of Sc131 genes were then used to predict biochemical pathways using the pathway tools. KEGG pathways and GO terms were retrieved from KEGG database (<http://www.kegg.jp/kegg>) and GO database (<http://geneontology.org>), respectively.

### Defining differentially expressed genes

To compare the gene expression level in different libraries, we evaluated repeat correlation of samples. The transcript level of each expressed gene was calculated and normalized to the Fragments Per Kilobase of exon model per Million mapped reads (FPKM). Differentially expressed genes (DEGs) under ethanol stress were screened out using the combined criteria of at least a twofold change and the value of  $q$  less than 0.05.

### Quantitative real-time PCR validation

Ten representative genes between the ethanol-GTP and the ethanol group were analyzed using real-time PCR method to evaluate the validity of Illumina analysis. Total RNA of Sc131 was reverse-transcribed into cDNA using Fastquant RT Kit (with gDNase) (TIANGEN BIOTECH). Then PCR reactions were carried out using a LightCycler96® (Roche Diagnostics GmbH, Mannheim, Germany) thermocycler with the following programs: 94 °C for 3 min, followed by 40 cycles of 95 °C for 3 s, 60 °C for 25 s, and a final extension of 72 °C for 2 min. The relative expression level of target genes was measured with the  $2^{-\Delta\Delta CT}$  method and ALG9 was used as the reference gene (Teste et al. 2009).

### Statistical analysis

Analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA, V 17.0.0). Comparison between groups was performed using one-way analysis of variance (ANOVA) with the post hoc Tukey test for multiple comparisons at the  $P < 0.05$  level which was considered statistically significant.

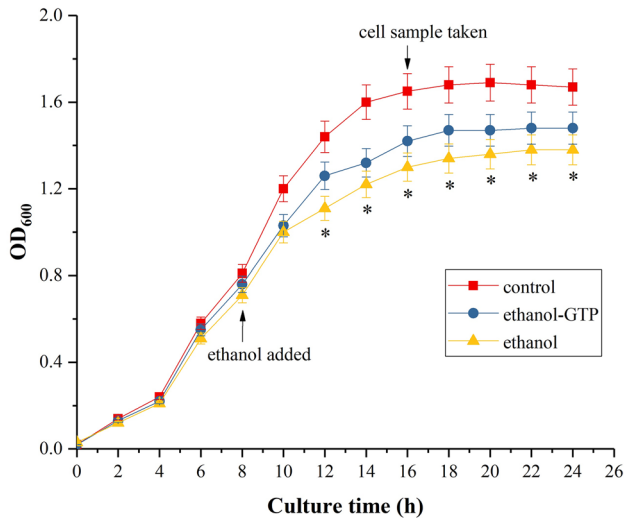
## Results

### Characterization of GTP

In the present study, we prepared GTP from green tea by column chromatography. From the HPLC chromatogram of the prepared GTP, EGC, C, theophylline, EGCG, GCG and ECG were identified according to the retention times and online DAD spectra of authentic standards. Here, we found that green tea from Ningbo contained relatively high content

**Table 1** The contents of tea catechins and theophylline in the prepared GTP

Components	Content (mg/g)
EGC	230.02 ± 12.42
C	5.37 ± 0.42
Theophylline	0.32 ± 0.01
EGCG	621.78 ± 13.78
GCG	29.87 ± 1.93
ECG	174.21 ± 8.32

**Fig. 1** Growth curves ( $OD_{600}$ ) of Sc131 with 10% (v/v) ethanol added at the exponential phase after cultivated for 8 h. Results are expressed as mean  $\pm$  SD ( $n=3$ ). \*Indicates significant differences ( $P<0.05$ ) between ethanol and ethanol-GTP group

of EGCG, and the content of tea catechins and theophylline in the prepared GTP is shown in Table 1.

### Illumina sequence data

Library representing transcriptome of each sample was constructed respectively for RNA-seq. In total, 22.74 Gb clean data were obtained, and the average quality value was  $\geq 30$  for more than 95% reads (Table S1). Quality screened reads were aligned and mapped to Sc131 reference genome using Tophat. The results showed that substantial proportion of reads of each library (more than 94%) was matched to Sc131 genes.

### Defining DEGs

As shown in Fig. 1, the growth of Sc131 cells was strongly inhibited by the ethanol interference at the exponential phase and the addition of GTP could effectively alleviate such ethanol stress. In addition, the addition of GTP alone did not significantly affect the growth of Sc131 (Fig. S2). Totally,

compared with the control group, 583 DEGs were identified in the ethanol group, including 216 up-regulated and 367 down-regulated genes. Moreover, 421 DEGs, including 178 up-regulated and 243 down-regulated genes were identified in the ethanol-GTP group in comparison with the ethanol group. Table S2 showed DEGs between the ethanol and the control group, while Table S3 showed DEGs between the ethanol-GTP and the ethanol group.

### GO classification analysis

In Fig. 2, GO analysis of DEGs between the ethanol group and the control group showed that after ethanol treatment, GO terms enriched in Biological Process domain included 'biological process', 'translation', 'cytoplasmic translation', 'transport', and 'transcription, DNA-templated'. For Cellular Component domain, the main enriched GO terms consisted of 'cytoplasm', 'nucleus', 'membrane', 'integral component of membrane', and 'mitochondrion'. In Molecular Function domain, the largest groups were 'molecular function', 'structural constituent of ribosome', 'metal ion binding', 'nucleotide binding' and 'hydrolase activity'. To gain insight into the function of GTP, we also analyzed the DEGs in the ethanol-GTP group compared with the ethanol group (Fig. 3). After GTP supplement, the DEGs in Biological Process domain were mainly distributed in the groups of 'biological process', 'transport', 'translation', 'cytoplasmic translation', and 'transcription, DNA-templated'. We observed a high percentage of genes assigned to 'cytoplasm', 'nucleus', 'membrane', 'mitochondrion' and 'integral component of membrane' in Cellular Component domain, while with respect to Molecular Function domain, most genes fell into 'molecular function', 'metal ion binding', 'structural constituent of ribosome', 'nucleotide binding' and 'RNA binding' groups. GO analysis of DEGs between the ethanol and the control group was shown in Table S4, while GO analysis of DEGs between the ethanol-GTP and the ethanol group was shown in Table S5.

### KEGG pathway analysis

KEGG pathway database that provides a molecular-level information about the molecular interaction, reaction and relation networks of the biological system, is valuable for our further understanding about the genetically and biologically complex behaviors. KEGG pathway enrichment analysis for DEGs between the ethanol group and the control group revealed the complicated pathways related to ethanol stress (Fig. 4a). Notably, 50 DEGs were annotated to 'Ribosome', which was the most enriched pathway, indicating the critical role of ribosome in response to ethanol stress. Considering ribosome was related with the translation from mRNA into protein, the enrichment of DEGs in ribosome

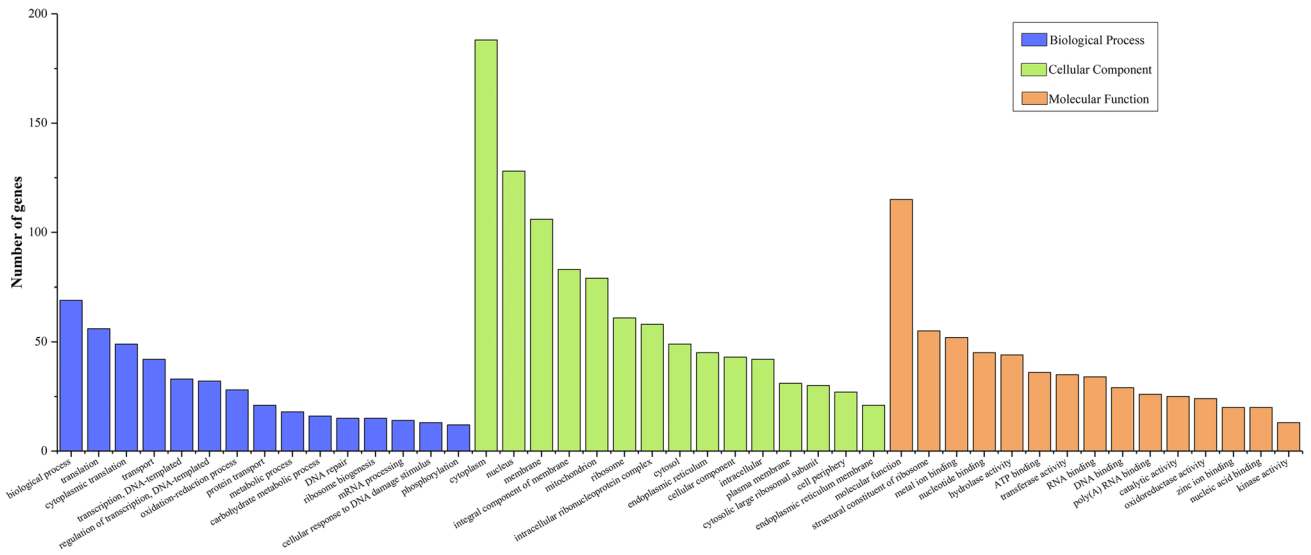


Fig. 2 GO analysis of DEGs between the ethanol and the control group

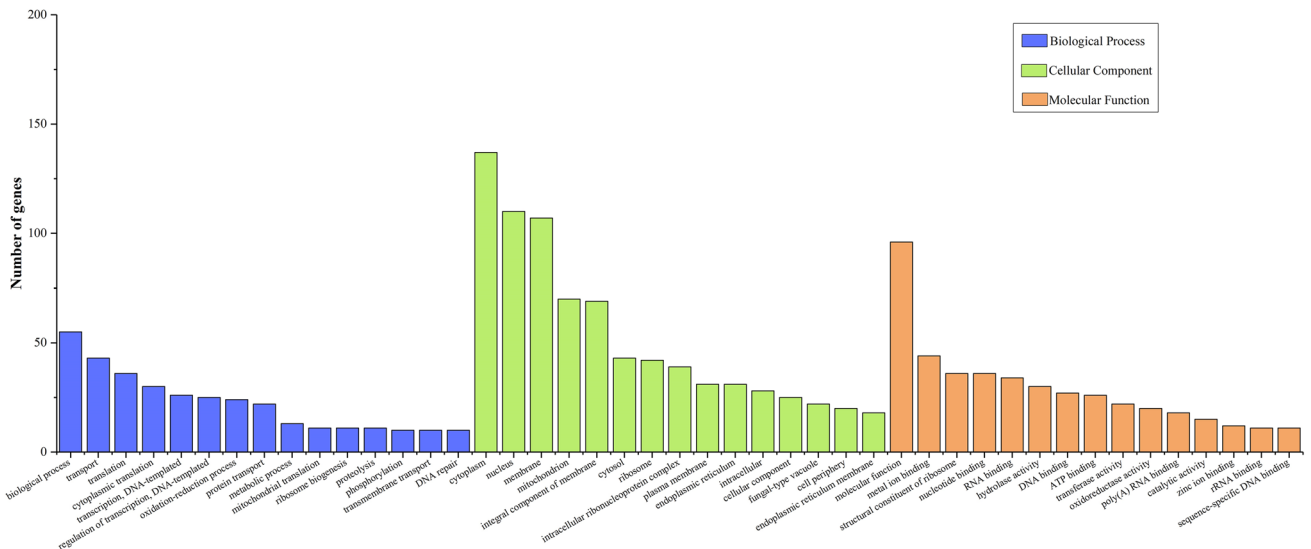
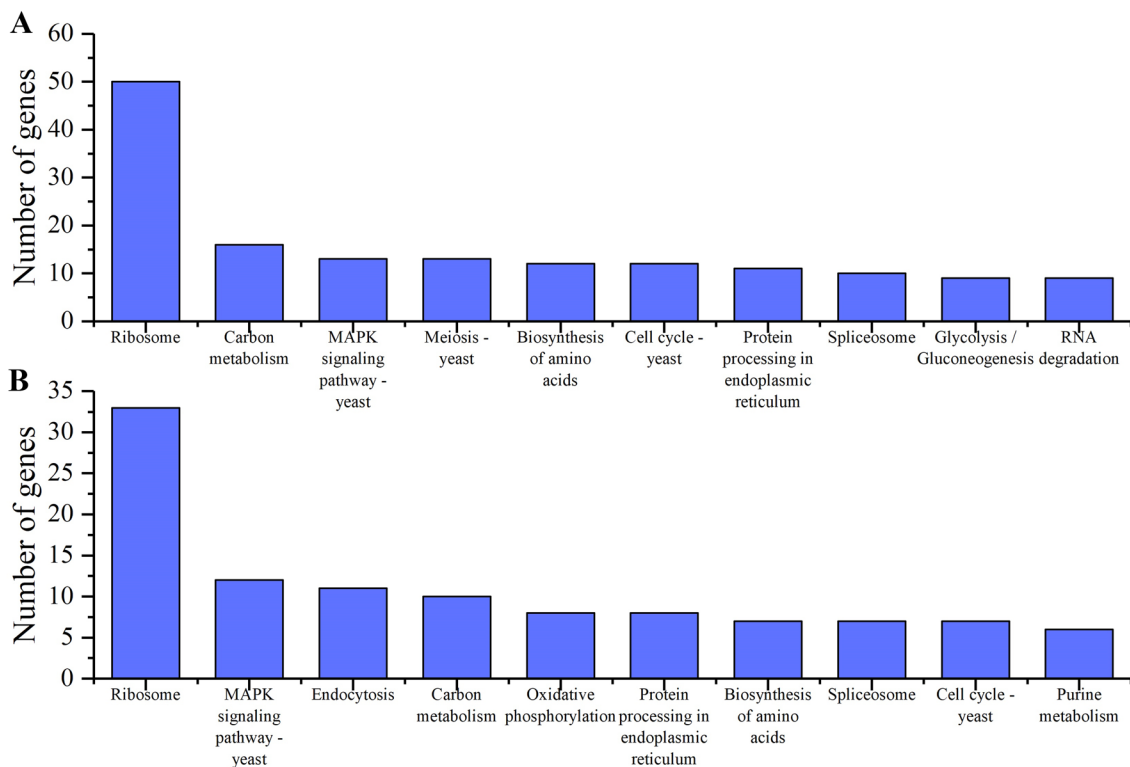


Fig. 3 GO analysis of DEGs between ethanol-GTP and the ethanol group

might imply a great influence of ethanol on enzymes, which was consistent with the biological process of GO analysis as summarized above. The gene expression related to ‘Carbon metabolism’ was also significantly changed with 16 DEGs annotated. Yang et al. (2012) indicated that ethanol affected carbon metabolism of *S. cerevisiae* through the reduction of mitochondrial membrane integrity. The addition of ethanol led to an obvious reduction in the mitochondrial membrane potential and a marked increase in the concentrations of intracellular reactive oxygen species (ROS). Then the ethanol-induced decrease of the proton motive force and the requirement of removing ROS resulted in an increased energy demand for cell maintenance (Yang et al. 2012). Such

energy demand induced the carbon flux to redistribute from mainly ethanol production to the citrate cycle (TCA cycle). 13 DEGs were annotated to ‘MAPK signaling pathway-yeast’ and ‘Meiosis-yeast’, respectively. It is possible that Sc131 entered sexual reproduction, underwent sporulation, and produced ascus and ascospore under ethanol pressure. Furthermore, we compared the DEGs between the ethanol-GTP group and the ethanol group (Fig. 4b). The pathways containing more than 10 DEGs were those associated with ‘Ribosome’ (30 DEGs), ‘MAPK signaling pathway-yeast’ (12 DEGs), ‘Endocytosis’ (11 DEGs) and ‘Carbon metabolism’ (10 DEGs). KEGG analysis of DEGs between the ethanol and the control group was shown in Table S6, while



**Fig. 4** KEGG analysis of DEGs between the ethanol and the control group (a) and between the ethanol-GTP and the ethanol group (b)

KEGG analysis of DEGs between the ethanol-GTP and the ethanol group was shown in Table S7.

### 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 10 candidate DEGs by RT-qPCR. These candidates included 5 up- and 5 down-regulated genes. The RT-qPCR results showed that, after the addition of GTP, the expression of *PST1*, *MID2*, *KRE1*, *AAT2* and *CHA1* were up-regulated, while *FLO10*, *FLO11*, *NTH1*, *MXR2* and *COX9* were down-regulated (Fig. S3). 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.

## Discussion

### Cell wall and membrane

In *S. cerevisiae*, the cell wall has a dynamic structure which contains  $\beta$ -(1,3)-,  $\beta$ -(1,6)-glucan, chitin, and heavily glycosylated mannoproteins (Klis et al. 2002). The polysaccharides are responsible for the structural function of the cell

wall, while the mannoproteins act as “fillers” and are crucial for the permeability of the cell wall. Disruption of the cell wall exerts a serious effect on the morphology and growth of *S. cerevisiae* cell, even rendering it susceptible to lysis and death (Ragni et al. 2011).

Compared with the control group, significantly up-regulated genes in the ethanol group related to cell wall structure included *SED1* for cell surface glycoprotein, *SPI1* for glycosylphosphatidylinositol (GPI)-anchored cell wall protein, *TIR1* for cell wall mannoprotein, *CCW12* for cell wall integrity and *HSP150* for cell wall organization. The proteins encoded by *SED1*, *SPI1* and *TIR* family and *CCW12* belong to GPI-anchored covalently linked cell wall proteins (GPI-CWPs) which are either structural proteins or enzymes involved in cell wall biogenesis (Ragni et al. 2011). The significantly differential expression of these genes might be due to the compensatory mechanism to counteract ethanol stress and protect the cell wall from damage caused by ethanol. However, some important genes, such as *PST1* related to mannoprotein biosynthetic, *MID2* implicated in cell wall organization and *KRE* family (*KRE1*, *KRE6* and *KRE29*) required for  $\beta$ -glucan synthesis were significantly down-regulated upon ethanol stress. GTP addition could relieve the negative effects induced by ethanol. Compared with the ethanol group, *PST1*, *MID2* and *KRE1* genes were significantly up-regulated in the ethanol-GTP group. In addition, *HVG1*

regulating the activity of guanosine diphosphate mannose (GDP-mannose) transporter was significantly up-regulated after the GTP intervention under ethanol stress, which was contrary to that in the ethanol group, indicating the benefit of GTP for the formation of mannose protein. Similarly, *CHS5*, a gene associated with chitin synthesis was also significantly up-regulated in the ethanol-GTP group. The effects of deletion of *CHS5* suggested that it was critical for the cell fusion step of mating (Santos et al. 1997). In the current study, we also observed the *FLO* genes (*FLO10* and *FLO11*) involved in the flocculation of *S. cerevisiae* cells were significantly up-regulated under ethanol treatment, indirectly indicating the adverse effect of ethanol on Sc131 cells. However, the administration of GTP under ethanol stress could induce the significant down-regulation of *FLO10* and *FLO11*. Besides flocculation, *FLO11* is involved in various cell properties, such as pseudohyphal mode of growth and formation of the air-liquid interfacial biofilm.

For *S. cerevisiae* cells, the membrane structure and function appear to be a predominant target of ethanol. In the domain of Cellular Component by GO classification analysis, many DEGs identified in association with ethanol tolerance were implicated in membrane. For example, *AGP1* for plasma membrane, *ETRI* for saturated fatty acid metabolism, *MCR1* for fatty acid, lipid, and isoprenoid metabolism were found to be significantly up-regulated in ethanol response. It is known that the increasing concentration of ethanol can gradually reduce cell viability by destroying the integrity and function of cell membrane during fermentation (Piper 1995). Ethanol at first destroys the normal membrane structures through increasing the fluidity of the plasma membrane. In response, yeast cells may alter membrane compositions to antagonize membrane fluidization and increase membrane stability. The level of ergosterol or unsaturated fatty acids (UFAs) changed in response to high concentration of ethanol (Alexandre et al. 1994).

Ergosterol is a major component of *S. cerevisiae* membrane, and essential for the normal structure and function of cell membrane through regulating the delicate balance among membrane components such as lipids and proteins (Bagnat et al. 2000; Shobayashi et al. 2005). Although higher level of ergosterol in yeast was associated with higher ethanol tolerance, the genes related to ergosterol biosynthesis, including *ERG2*, *ERG3*, *ERG5*, *ERG6*, *ERG24* and *ERG28* did not show significant difference between the ethanol group and the control group. With regard to UFAs, *TES1* related to biosynthesis and regulation of UFAs was significantly up-regulated in the ethanol group, which might compensate the deficits caused by ethanol stress. The reason for the increase of UFAs under ethanol stress was suggested to antagonize the stereo-chemical effect from the quite hydrophilic ethanol on the head groups of the phospholipid bilayer (Weber and de Bont 1996). Additionally, many other

important genes, such as *ENO1*, *HSP30* and *HOR7* involved in the membrane composition and organization were significantly down-regulated under the ethanol challenge. However, these genes showed opposite trends in the ethanol-GTP group, suggesting the alleviation effect of GTP.

## Basic metabolism

A number of genes related to basic metabolism in *S. cerevisiae* were identified in this study. Amino acids, as the major metabolites, are associated with numerous cellular biosynthetic and metabolic processes. Takagi et al. (2005) found that the intracellular accumulation of proline could improve the ethanol tolerance of *S. cerevisiae*. In the present study, four significantly down-regulated genes (*AAT2*, *CHAI1*, *FCY1*, *MSC7*) related to the metabolism and biosynthesis of proline were observed in the ethanol group compared with the control group, suggesting that metabolism and biosynthesis of proline were suppressed by the ethanol treatment. However, these genes were all significantly up-regulated in the ethanol-GTP group in comparison with the ethanol group. In addition, *CTT1* and *CCP1* related to the tryptophan metabolism process were significantly up-regulated, while *PDC1* encoding the tryptophan catabolic process was significantly down-regulated under ethanol stress. The role of tryptophan biosynthesis in enhancing ethanol stress tolerance of *S. cerevisiae* was unclear up to now, but a previous research pointed out strains overexpressing tryptophan biosynthesis or permease genes exhibited higher tolerant levels to ethanol (Ma and Liu 2010).

Numerous DEGs of carbohydrate metabolism were also identified in our study. Trehalose, an important reserve carbohydrate and compatible solute in *S. cerevisiae*, is considered to help *S. cerevisiae* to deal with ethanol stress and other stresses (Ogawa et al. 2000). Two hypotheses, the glass transition hypothesis and the water replacement hypothesis, have been proposed to explain the protective effect of trehalose at the molecular level (Ma and Liu 2010). Trehalose is synthesized from uridine 5'-diphosphate-glucose and glucose-6-phosphate (glucose-6P) in a two-step reaction required for trehalose-6P synthase encoded by *TPS1* and trehalose-6P phosphatase encoded by *TPS2*. A previous study stated that the second step might be a bottleneck of the trehalose biosynthesis pathway (Mahmud et al. 2010). Therefore, overproduction of Tps2p protein resulted from *TPS2* gene overexpression was critical to trehalose accumulation. The down-regulated *TPS2* in the ethanol group revealed that the trehalose biosynthesis might be negatively influenced by high level of ethanol. On the contrary, *TPS2* was significantly up-regulated when GTP was added, which might contribute to better growth of *S. cerevisiae* under ethanol stress. Interestingly, *NTH1* gene involved in trehalose catabolic process was significantly down-regulated in the



ethanol-GTP group. The co-induction of trehalose degradation genes was confirmed by previous literature (Ma and Liu 2010). As persistence of high trehalose concentration might inactivate some important yeast enzymes, the co-operation of trehalose synthesis and degradation genes could mediate an optimal trehalose level in the cell for its proper functions in response to environmental stresses. Besides, as glycogen and trehalose pathways are very close, biosynthesis and catabolism of trehalose may be subtly affected by glycogen metabolism. Two genes (*HSP30* and *HSP42*) encoding heat shock proteins (HSPs) were significantly down-regulated in the ethanol group, but up-regulated in the ethanol-GTP group. HSPs are the most common protective proteins which have been reported to work with trehalose in response to stress stimuli in *S. cerevisiae* cells (Li et al. 2017). HSPs have also shown to protect cells against oxidative stress (Martindale and Holbrook 2002). Moreover, many DEGs involved in glycolysis, which is a crucial pathway in *S. cerevisiae* metabolism and the fermentation of ethanol were observed in our study. In the ethanol group, *RBK1* involved in the process of converting glucose to glucose-6P, *TDH2* associated with the conversion from glycerate-2P to pyruvate, *PDC1* regulating the pyruvate kinase activity in the conversion of pyruvate to acetaldehyde, *MSC7* related to the aldehyde dehydrogenase activity in the conversion of acetaldehyde to acetate, and *ADH1* required for the process from acetaldehyde to ethanol were all significantly down-regulated comparison with the control group. High level of ethanol treatment suppressed ethanol biosynthesis. Two genes, *IMA1* and *MAL12*, involved in starch and sucrose metabolism were also significantly down-regulated. However, the addition of GTP could induce these genes significantly up-regulated, suggesting that GTP was a potential functional supplement for the industrial ethanol production. Lipid metabolism of *S. cerevisiae* cells was also influenced by ethanol stress. *YAT1* related with lipid metabolic process was significantly down-regulated in the ethanol group and showed an opposite trend by GTP intervention.

### Redox regulation

ROS are highly reactive and capable of damaging a wide variety of cellular components, such as DNA, lipids, proteins and other substances in cells. *S. cerevisiae*, like other organisms, has evolved a few effective antioxidant defense systems to detoxify ROS (Morano et al. 2012). In the present study, we found most oxidative stress-related genes, such as *CTT1* for cytosolic catalase T, *SOD1* for superoxide dismutases, *GLR1* for reduced glutathione and *TRX3* for thioredoxin in the mitochondrial thioredoxin system, were significantly up-regulated in the ethanol group, which implied yeast cells exhibited increased ROS levels during exposure to high ethanol stress. However, two

thioredoxin-encoding genes *TRX1* and *TRX2* involved in the cytoplasmic thioredoxin system did not recognized as significant difference. Thus, it can be inferred that ethanol stress mainly affected mitochondrial thioredoxin systems, rather than cytoplasmic thioredoxin systems. The redox states of the cytoplasmic and mitochondrial thioredoxin systems are independently maintained (Trotter and Grant 2005). Other genes (*MXR2*, *COX9*, *ETR1* and *SDH3*) involved in oxidation–reduction process and mitochondrial respiratory chain were also significantly up-regulated in the ethanol group.

Oxidative stress has been thought as one of the major molecular mechanisms involved in ethanol toxicity. GTP is a well-known antioxidant, having a strong ability to suppress accumulation of ROS. However, few studies reported the protective effect of GTP against ethanol-induced stress in yeast. In the ethanol-GTP group, we observed those genes implicated with the generation of ROS or mitochondrial electron transport were down-regulated. Previous researches revealed that microbes in the exposure to polyphenols up-regulate proteins involved in defensive mechanisms, while simultaneously down-regulate proteins associated with metabolic and biosynthetic process (Cardona et al. 2013). Catechins were thought to be able to influence cells not only by antioxidant properties, but also by interacting with signaling pathways involved in other important bioprocesses (Monagas et al. 2010). It was worth noting that *MID2* associated with response to osmotic stress was up-regulated, indicating that GTP might also benefit for *S. cerevisiae* to cope with ethanol-induced osmotic stress.

### Conclusions

In this study, the influence of GTP, as well as ethanol, on Sc131 cells was investigated by RNA-seq transcriptomic analysis. The increasing concentration of ethanol during the process of fermentation is the major stress affecting the yeast cells and may lead to negative consequences of ethanol yields and yeast viability, while GTP was found to exert ameliorate effect on Sc131 under the ethanol stress. The present study provided precise and comprehensive information on the level of transcripts for *S. cerevisiae* in response to ethanol stress and GTP intervention, which may promote the development of bioethanol production industry. Further investigations are needed to determine the exact mechanism of GTP protective effect on *S. cerevisiae* during fermentation.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no competing financial interest.

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