Transcriptome research on differentially expressed genes in a thermotolerant yeast strain from Daqu of Luzhou-flavor liquors

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Abstract

Background: As for thermotolerance of yeasts, early research focuses on comparative biology. However, there is rare research on genes related to thermotolerance of yeasts.Morphological observation, trehalose concentration, and cellular structure of a thermotolerant yeast strain were basically studied. Together with RNA-Seq, the differentially expressed genes (DGEs) related to the thermotolerance of the liquor-making yeast strain at 28, 37 and 43 ºC were screened out at the transcriptome level. It was found trehalose concentration increment can improve thermotolerance,and the destruction of strain cellular structure led to a decline in thermotolerance.

Results: After treatment of the thermotolerant yeast strain at 28 ºC compared with 37 ºC, 517 DGEs were screened out, including 435 upregulated genes and 82 downregulated genes. After treatment at 37 ºC relative to 43 ºC, 632 DGEs were recognized, including 393 upregulated genes and 239 downregulated genes.

Conclusions: By combining physicochemical property analysis and transcriptome analysis, this preliminary study uncovers the DEGs responsible for the thermotolerance of a thermotolerant yeast strain, and theoretically underlies further research on the high temperature tolerance of liquor-making yeasts.

Introduction

Daqu is mainly produced from wheat, barley, peas and broomcorn through grain wetting, crushing, mixing, trampling for shape forming, and natural inoculated fermentation[1]. Daqu provides abundant flavor compounds and precursor substances for liquor making[2]. It is rich in various enzymes and microbes, and these microbes participate in the saccharification, liquor formation and aromatic generation of liquor fermentation, thereby affecting the quality of finished liquors[3]. Yeasts are major functional microbes of Daqu[4], and play critical roles in starch saccharification, alcohol generation, aromatic substance formation, fermentation by fermented grains, and product quality[5].

The optimal fermentation temperature of conventional liquor-making yeasts is 28–33 ºC and usually does not exceed 36 ºC. High summer temperatures interfere with normal vital activities of yeasts, leading to premature senility, sometimes industrial fermentation cannot be completed or progress is slow [6–7]. As a result, summertime yield/quality decline of Daqu occurs every year, so many liquor plants choose depressurization in summer, which raises liquor-making costs[8]. Yeast with high temperature tolerance can effectively adapt to high temperature environment [9–10]. H ence, studying the high temperature resistance of yeast strains is very significant for the production activities of liquor plants.

As for thermotolerance of yeasts, early research focuses on comparative biology. Specifically, the thermotolerant traits are interpreted by comparing the enzyme proteins[11], nucleic acids[12], cell membrane structures & functions[13], and synthetic biology systems[14] between thermophilic yeasts and medium-temperature yeasts[15]. However, there is rare research on genes related to thermotolerance of

yeasts. We think the combination of multi-omics methods (e.g. transcriptomics) will theoretically underlie research on the thermotolerant ability of yeast strains when we study the genetic transcriptiion, transcription and regulation rules if the cells are under a certain function on the whole[16–19].

In this study, phenotype analysis, culture medium composition, and strain cellular structures were studied to explore the influence factors on thermotolerance of yeast strains. Then together with transcriptomics, the thermotolerance formation mechanisms was explained and uncovered preliminarily.

Materials And Methods

Materials and reagents

The strain

The tested thermotolerant yeast strain was *Pichia kudriavzevii* (ZG-3) preserved at Henan Liquor Style Technology Research Center, Henan University of Animal Husbandry and Economy.

Reagents

Glucose, agaragar (Tianjin Kemiou Chemical Reagent Co. Ltd.); peptone, trehalose (Beijing Abxing Biotech Co. Ltd.); yeast powder (UK Oxoid); KH_2PO_4 (Tianjin De'en Chemical Reagent Co. Ltd.); NaCl (Xilong Chemical Co. Ltd.); Trizol kits (US Invitrogen); $CaCl_2$, CCl_3COOH, $(NH_4)_2SO_4$ (Zhengzhou, Paini Chemical Reagent Plant); $MgSO_4$, sodium citrate (Sinopharm Chemical Reagent Co., Ltd); double distilled water (Shanghai Sangon Biotech Co. Ltd.); anthrone (Beijing Solarbio Co. Ltd.) were used here.

Culture media

Liquid culture medium (pH 4.5): 20.0 g of glucose, 20.0 g of peptone, and 10.0 g of yeast powder were dissolved in 1000.0 mL of water, and sterilized at 121 ºC for 15 min.

Solid culture medium YPD: medium (natural pH): 10 g of yeast powder, 20 g of tryptone, and 20 g of anhydrous glucose were dissolved in 1000 mL of water, and sterilized at 115 ºC for 15 min[20].

Fermentation medium: 20 g of peptone, 10 g of yeast extract powder, 100 g of glucose, 1 g of $MgSO_4$, 1 g of $(NH_4)_2SO_4$, and 1 g of KH_2PO_4 were diluted by distilled water to 1000 mL (natural pH) and sterilized at 121 °C for 20 min[21].

Instruments

A DRP-9052 thermostatic oscillation incubator (Shanghai Senxin Laboratory Instrument Co. Ltd.), an SW-CJ-2FD double two-sided purification workbench and a Sigma high-speed freezing centrifuge (Suzhou Purification Equipment Co. Ltd.), An LDZX-50FBS high-pressure steam sterilization pot (Shanghai Shenan Medical Instrument), a BCD-216SDN refrigerator (Qingdao Haier Co. Ltd.), an A590 ultraviolet

spectrophotometer (Shanghai Aoyi Instrument Co. Ltd.), and a CX31 biological microscope (Guangzhou Olympus Co. Ltd.) were used here.

Phenotypes of the thermotolerant yeast strain

Activation of the thermotolerant yeast strain

The strain was taken out from the refrigerator at -80 ºC, and recovered from the preservation state. It was inoculated on a superclean bench. The inoculated culture medium was put into a thermostatic incubator at 28 ºC, until milk white colonies appeared. Then the colonies with representative characteristics of yeast strain colonies were selected, and lineated twice. Only the colonies microscopically examined to be pure were used in subsequent experiments[22-23].

Macromorphology of the thermotolerant yeast strain

From the activated strain, single colonies were selected and lineated. After cultivation in a yeast incubator at 28 ºC for 48 h, colonial morphology was observed[24]. Then after cultivation in a thermostatic shaking table at 28 ºC for 24 h, the strain was thermalactivated at 43 ºC for 24 h. The strain was put back to the thermostatic shaking table at 28 ºC for 24 h of recovering. The above steps were repeated, during which the strain was photographed to record the morphological changes before and after thermalactivation.

Micromorphology of the thermotolerant yeast strain

The strain was dyed by cultivation with methylene blue at 28 or 43 ºC and then microscopically observed.

Basic research on the thermotolerant yeast strain

Effects of medium components on thermotolerant ability of the yeast strain

Effects of peptone and yeast extractum on thermotolerant ability of the yeast strain

The thermotolerant yeast strain treated at 43 ºC was inoculated at the 5% amount into 10, 15, 20, 25, 30 g/L YEPD liquid media, which were cultured at 43 ºC and 150 r/min for 48 h[25]. Then the absorbance of each bacterial solution at λ=600 nm was measured. With the same method, the thermotolerant ability at different concentrations of yeast powders was detected.

Effects of inorganic salt ions on thermotolerant ability of the yeast strain

The thermotolerant yeast strain treated at 43 ºC was inoculated at the 5% amount into 0.5, 1, 1.5, 2, 2.5, 3 mmol/L $CaCl_2$ YEPD liquid media, which were cultured at 43 ºC and 150 r/min for 48 h[25]. Then the absorbance of each bacterial solution at λ=600 nm was measured. A system without adding inorganic salt ions was set as the control. With the same method, the effects of $ZnSO_4$, $MgSO_4$, NaCl, KH_2PO_4 at different concentrations on the thermotolerant ability were detected.

Association between strain cell structure and thermotolerance of yeast

Association between trehalose concentration and thermotolerant resistance of yeast

Fucose concentrations were measured by anthrone colorimetry[26]. Li Yanling et al. [27]compared three sulfuric acid - anthrone colorimetric methods, and found sulfuric acid - anthrone dilute sulphuric acid method was the most effective and economical. The specific conditions were: 85% H_2SO_4, 1.0 mL of trehalose solution, 3.0 mL of anthrone sulphuric acid reagent, boiling coloration 10 min, and absorbance at 625 nm.

Shang Lei [28] used a pipette to accurately suck 0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0 mL of a standard trehalose solution into 7 test tubes, which were all diluted with distilled water to 2.0 mL and then added with 4.0 mL of a newly-prepared sulfuric acid - anthrone reagent. The first tube served as a blank. Then the absorbance at 625 nm was detected. A standard curve was plotted with the absorbance as Y-axis and trehalose concentration as X-axis.

The yeast strain was cultured at 28 or 43 ºC for 24 h. Then the fermentation culture solutions (each 5 mL) were centrifuged at 2000 rpm/min for 5 min, washed with deionized water that was placed in an ice-bath in advance, and centrifuged again. Then 4 mL of a 0.5 mol/L CCl_3COOH solution was added. In the resulting solutions, when only trehalose existed and after oscillation to be uniform, the solutions were put into an ice water mixture with ice cubes for extraction. Oscillation was operated every 15 min. After 1.5 h, the solutions were centrifuged at 950 rpm/min for 5 min. Then the supernate (1 mL) was collected and added with 3.0 mL of the sulfuric acid - anthrone reagent, followed by measurement of optical density (OD)[28].

Association between cell membrane permeability and thermotolerance of yeast

The thermotolerant yeast strain treated at 43 ºC was inoculated by a 10% amount into the YEPD medium for cultivation. Samples were collected every 1 h. With the yeast strain treated at 28 ºC and other similar conditions as the control, extracellular nucleotide concentration of the strain was measured.

In brief, each sample was washed with double distilled water until the absorbance at λ=260 nm of the supernatant was nearly 0. Then the cleaned strain was removed to an alcohol buffer solution (pH 5.0 and containing 0.1 mol/L sodium citrate), which was put into an oscillation incubator for culture. Samples were collected every 12 h. Then absorbance values of each sample at λ=260 nm and 280 nm were measured until the two absorbance values both reached the corresponding average values[25]. The extracellular nucleotide concentration of the strain upon sampling was Ce ($\mu g \cdot mL^{-1}$)=[(OD_{260}×11.87 - OD_{280}×10.40)×9]/100.

Transcriptomics research on thermotolerant yeast

Sample collection

ZG-3 was activated and then cultured at 28 ºC for 3 d. An appropriate amount of each sample was collected, in which the count of fresh yeast was no less than 6×10^5 cells. After raising to 37 ºC, cultivation was continued for 24 h. Then an appropriate amount of the sample was collected. The temperature was raised to 43 ºC, and the final sampling was collected. The three times of sampling were named Sce28, Sce37, and Sce43 respectively. Then the strain was washed with 5 mL of sterile water 1-3 times, and then removed to a 1.5 mL or 2.0 mL centrifuge tube, which was centrifuged at 4 ºC for 10 min (1500 $r\cdot min^{-1}$). The supernatant was removed, and the sediment strain was reserved, which was immediately deepfrozen by liquid nitrogen[29]. After that, each of the three samplings was sent to RNA extraction.

Total RNA extraction

Transcriptome sequencing was conducted in Guangzhou Genedenovo. Specifically, the qualified samples were sent to total RNA extraction using Trizol kits. RNA quality was assessed on Agilent 2100 Bioanalvzer, and examined by RNase-free agarose gel electrophoresis.

Library construction and RNA-Seq

After extraction of total RNA, eukaryotic mRNA was enriched using Oligo(dT) beads, and rRNA was removed using a Ribo-ZeroM magnetic kit to enrich prokaryotic mRNA. Then the enriched mRNA fragments were cut using a fragment buffer solution into short fragments, and reverse-transcribed using random primers into cDNA. Two-chain cDNA was synthesized using DNA polymerase I, RNase H. dNTP, and a buffer. Then the cDNA fragments were purified using a QiaQuick PCR extraction kit, followed by terminal restoration, A base addition, and connection to an Illumina sequencing adapter. The connected products were screened by agarose gel electrophoresis, amplified by PCR, and sequenced using Illumina Novaseq6000 (Gene Denovo Biotechnology).

Filtering and processing of original sequencing data

The reads from the sequencing machine included the original data of low-quality bases, which will interfere the subsequent assembly and analysis. Hence, to acquire high-quality clean data, we filtered the data on Fastp 0.18.0[30]. The parameters were set as follows: 1) the reads of inset, 2) reads containing > 10% unknown nucleotides (N) and 3) low-quality reads with > 50% low-quality (Q20) bases were removed.

On the comparison tool Bowtie2 2.2.8[31], the reads were mapped to a ribosome RNA (rRNA) database. The rRNA-mapped data were deleted, and the remaining clean data were used for subsequent assembly and gene abundance computation. Then after comparison with the reference genome, an index of reference genome was established. The clean reads at the paired ends were mapped on HISAT 2.2.4 onto the reference genome[32]. Based on the reference genome sequence, the mapped reads were spliced on StringTie, and compared with the original genome annotations. The raw counts were statistically analyzed on DESeq2, and at the condition of $P<0.05$ & $|\log2FC|\geq2$, the differentially expressed genes (DEGs) between the test group and the blank group were screened out.

Results

Phenotypes of the thermotolerant yeast strain

Colonies of the thermotolerant strain ZG-3 were observed (Fig. 1). For the strain treated at 28 ºC, the colonies were large with smooth surface and were round-shaped without folds (Fig. 1a). Microscopic observation showed the strain was mostly ovate- or round-shaped (Fig. 1b). For the strain treated at 43 ºC, the colonies were smaller with smooth surface (Fig. 1c). Microscopic observation showed the colonies were nearly ovate-shaped with unilateral or many-sided budding, and was mostly dead (Fig. 1d).

Effects of physicochemical factors on thermotolerant ability of the yeast strain

Effects of peptone and yeast powder on thermotolerant ability of the yeast strain

Table 1
OD values of peptone and yeast powder at 600nm with different concentrations

	10 g/L	15 g/L	20 g/L	25 g/L	30 g/L
peptone	1.293	1.368	1.670	1.461	1.551
yeast powder	1.442	1.580	1.932	1.740	1.506

Results of single-factor control tests are listed in Table 1. Generally, the OD first rises and then declines with the increasing peptone concentration, and recovers at the level of 30 g/L, but is yet lower than the OD at 20 g/L, indicating the bacterial density maximizes and the high-temperature resistance ability is optimized at the peptone concentration of 20 g/L. Similarly, the bacterial density in the medium is maximized at the yeast powder concentration of 20 g/L, at which the high-temperature resistance ability is high.

Effects of inorganic salt ions on thermotolerant ability of the yeast strain

Table 2
ODs of inorganic salt ions with different concentrations at 600 nm

	0.5 mmol/L	1.0 mmol/L	1.5 mmol/L	2.0 mmol/L	2.5 mmol/L
NaCl	1.730	1.463	1.393	0.622	0.372
KH_2PO_4	0.508	0.853	1.440	0.555	0.386
$MgSO_4$	0.485	0.445	0.521	0.335	0.261
$CaCl_2$	0.421	0.630	0.693	0.575	0.318

Results of single-factor control tests are listed in Table 2. Generally, the OD first rises and then declines with the increasing NaCl concentration, and maximizes at the level of 0.5 mmol/L, indicating the bacterial density maximizes and the high-temperature resistance ability is excellent at this concentration. Similarly, the bacterial density in the medium is maximized at the level of 1.5 mmol/L KH_2PO_4, 1.5 mmol/L $MgSO_4$, or 1.5 mmol/L $CaCl_2$, at which the high-temperature resistance ability is high.

Association between strain cell structure and thermotolerance of yeast

Association between trehalose concentration and thermotolerance of yeast

The standard curve of trehalose concentration measured by the sulfuric acid-anthrone method is plotted in Fig. 2. Clearly, the regression equations of treatments at 43 and 28 ºC are y = 0.2068x-0.1489, R^2 = 0.9939 and y = 0.181x-0.2359, R^2 = 0.9621 respectively, where x is trehalose concentration, y is OD, and R^2 is correlation coefficient. Results suggest both equations show obvious linearity.

The ODs after treatments at 28 and 43 ºC are 2.795 and 3.398 respectively, which correspond to trehalose concentrations of 14.24 and 20.08 mg/mL respectively. The trehalose concentration after treatment at 43 ºC is significantly larger compared with the condition at 28 ºC, which preliminarily indicates the trehalose concentration is correlated with the thermotolerant ability of the yeast strain: a higher trehalose concentration reflects a higher thermotolerant ability.

Association between cell membrane permeability and thermotolerance of yeast

OD was substituted into computations: $Ce(\mu g \cdot mL^{-1})=[(0.913\times11.87-0.593\times10.40)\times9]/100 = 0.4203\ \mu g \cdot mL^{-1}$ (43 ºC); $Ce(\mu g \cdot mL^{-1})=[(0.134\times11.87 - 0.117\times10.40)\times9]/100 = 0.03364\ \mu g \cdot mL^{-1}$ (28 ºC).

Results suggest the extracellular nucleotide concentration in the yeast strain treated at 28 ºC is smaller than after the treatment at 43 ºC. Hence, higher temperature will moderately destroy cell membranes, leading to loss of intracellular nucleotides. Thus, the yeast strain treated at 43 ºC was more affected by high temperature. In other words, after cultivation at higher temperature, the cell membrane permeability was weakened, which was negatively correlated with the thermotolerant ability.

Transcriptomics analysis of thermotolerant yeast

Quality assessment and analysis of sequencing data

To explore the thermotolerant mechanism of the yeast strain under different culture conditions, we sequenced Sce28, Sce37, Sce43, and filtered the original sequences, which yielded 40492496, 43936966, and 43102251 clean sequences respectively. The average Q20 values of Sce28, Sce37 and Sce43 are 96.93%, 96.79% and 96.86% respectively, and the average Q30 values are 91.60%, 91.28% and 91.43% respectively (Table 3). Besides, the GC relative contents are 42.92%, 43.08% and 44.37% respectively, the base percentage Q20 values are all larger than 96.95%, and the base percentage Q30 values are all higher than 91.28%, indicating the transcriptome sequencing data are of high quality and accuracy and can be used into subsequent analysis.

Table 3
Transcriptome sequencing data and quality statistics

	CleanData	CleanData(bp)	Q20/%	Q30/%	GC content /%
Sce28	40492496	6073874400	5835229377 (96.93%)	5514220622 (91.60%)	2583903553 (42.92%)
Sce37	43936966	6590544900	6311069934 (96.79%)	5951504819 (91.28%)	2809141417 (43.08%)
Sce43	43102251	6465382700	6145500011 (96.86%)	5801075589 (91.43%)	2815240479 (44.37%)

Between-sample correlation analysis

The reliability of experimental results and the operational stability were assessed using Pearson's correlation coefficients (R^2). In the range of 0–1, R^2 closer to 1 indicates higher repeatability and similarity between two parties. Our results show the R^2 between any 2 of the 6 samples minimizes to 0.878, indicating our results are reliable and the sample selection is reasonable(Fig. 3).

Screening of DGEs

For the same organism, the expressions of some genes may significantly differ among different time points and among different environmental conditions. Similarly, when yeast strains migrate from the suitable temperature to a higher temperature, they regulate the expressions of certain genes in vivo to adapt to the higher temperature. The present study involved biologically repeated samples, and their gene differences were analyzed on DESeq2 v1.6.3 from Bioconductor. The setting was $P \leq 0.05$ and up-/down-regulation fold change ≥ 2.0. From Sce28 and Sce37, totally 517 DGEs were identified, including 435 upregulated genes and 82 downregulated genes, which account for 87.14% and 22.86% of total genes respectively. From Sce28 and Sce37, totally 632 DGEs were identified, including 393 upregulated genes and 239 downregulated genes, which account for 62.18% and 37.82% of total genes respectively (Fig. 4). After gene expressions among different samples are clustered, the genes showing similar expression patterns are considered to be functionally similar. Hence, the expression patterns of DGEs under different experiment conditions can be judged by clustering analysis, and thereby the functions of unknown genes can be deduced. Herein, differential analysis was conducted on DESeq2 at $P \leq 0.05$ and up/down-regulating fold change ≥ 10.0. Totally 517 significant DGEs between 28 and 37 ºC and 632 significant DGEs between 37 and 43 ºC were identified, which were used to plot gene heatmaps (Fig. 5).

GO enrichment analysis of DGEs

The DEGs as-identified were sent to GO enrichment analysis, from which information about participation in biological processes, cellular components, and molecular functions was identified. Thus, the DEGs were analyzed by GO function significance enrichment to clarify which DEGs were significantly correlated with biological functions.

The enrichment analysis of the DGEs in the thermotolerant yeast strain cultured under different temperatures is illustrated in Fig. 6. Results uncover 49 function groups, which are mainly annotated as bioprocesses, molecular functions, and cell components. The bioprocesses involve 25 subclasses, mainly include cell transformation, metabolic processes, biological modulation, and proliferation regulation. The molecular functions include 12 subclass that are mainly annotated as binding energy, catalytic activity, transport movement, and molecular function supervision. The cell components have 12 subclasses: cells, cellular components, organelles, and cell membranes.

KEGG enrichment analysis of DGEs

KEGG is a major public database of relevant gene pathways, and can be used to further study the complex biological behaviors of certain genes. The biological functions of organisms are mainly realized through intercoordinated cooperation between genes. Hence, DEGs can be utilized to analyze significance of pathways. Based on KEGG annotations, the gene metabolic pathways can be annotated to determine the dominant biochemical pathways and signal transduction pathways that involve the DEGs.

The enrichment bubble charts of 28 and 37 ºC show that 132, 118, 93, 91, 69, 67 and 64 genes are enriched in Cell cycle, Cell cycle process, Reproduction, Reproductive process, Mitotic cell cycle, Mitotic cell cycle process, and Meiotic cell cycle respectively (Fig. 7). The enrichment bubble charts of 37 and 43 ºC show that 124, 56, 45, 72, 82, 82, 74, 85 and 54 genes are enriched in Small molecule metabolic process, Drug metabolic process, Anatomical structure development, Oxidation-reduction process, Cell cycle, Phosphate-containing compound metabolic process, Cell cycle process, Phosphorus metabolic process, Response to chemical, and Regulation of biological quality respectively.

Conclusions

Basic research and transcriptomics research were combined to explore the DEGs related to the thermotolerant ability of a thermotolerant yeast strain in Daqu of Luzhou-flavor liquors. Results showed the macromorphology and micromorphology of this strain were both moderately altered under thermalactivation. When medium components (e.g. peptone, yeast powder) were changed, the thermotolerant ability of this strain was improved and was maximized at the level of 20 g/L. Inorganic salt ions also affected the thermotolerant ability, which was well improved at the level of 0.5 mmol/L NaCl, 1.5 mmol/L KH_2PO_4, 1.5 mmol/L $MgSO_4$, or 1.5 mmol/L $CaCl_2$. The trehalose concentrations after treatment at 28 and 43 ºC were 14.24 and 20.08 mg/mL respectively, which were increased and indicate an improvement of the thermotolerant ability. The extracellular nucleotide concentrations after treatment at 28 and 43 ºC were 0.4203 and 0.03364 $\mu g \cdot mL^{-1}$ respectively, indicating the cell membrane permeability and thermotolerant ability were weakened.

The transcriptomes of the thermotolerant strain treated at different temperatures (28, 37, 43 ºC) were sequenced on Illumina Novaseq, which showed the sequencing was of high quality. At $P \leq 0.05$ and up-/down-regulation fold change ≥ 2.0, after treatment at 28 ºC compared with 37 ºC, 517 DGEs were

screened out, including 435 upregulated genes and 82 downregulated genes. After treatment at 37 ºC compared with 43 ºC, 632 DEGs were screened out, including 393 upregulated genes and 239 downregulated genes. GO enrichment analysis of DGEs uncovers the DEGs in bioprocesses, molecular functions and cell components of the thermotolerant yeast strain after treatment at different temperatures. The DEGs were processed by KEGG enrichment analysis to determine the significant enriched pathways, and to illustrate the results on enrichment bubble charts. Comparison between treatments at 28 and 37 ºC shows the DEGs are significantly enriched in seven KEGG metabolic pathways: Cell cycle, Cell cycle process, Proliferation, Proliferation process, Mitotic cell cycle, Mitotic cell cycle process, and Meiotic cell cycle. Comparison between treatments at 37 and 43 ºC shows the DEGs are significantly enriched in 10 KEGG metabolic pathways: Small molecule metabolic process, Drug metabolic process, Anatomical structure development, Oxidation-reduction process, Cell cycle, Phosphate-containing compound metabolic process, Cell cycle process, Phosphorus metabolic process, Response to chemical, and Regulation of biological quality. Our findings offer some theoretical reference for further research on the DEGs related to the thermotolerant ability of thermotolerant yeast strains.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated in the current study are available in the NCBI BioProject database, accession # PRJNA 810519.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y.L.and C.P. conceived and designed the experiments. Y.L., W.G., H.L., and Z.Z. performed the experiments. S.H., X.W., and X.S. analyzed the data. Y.L. wrote the manuscript. C.P. revised manuscript.

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Figures

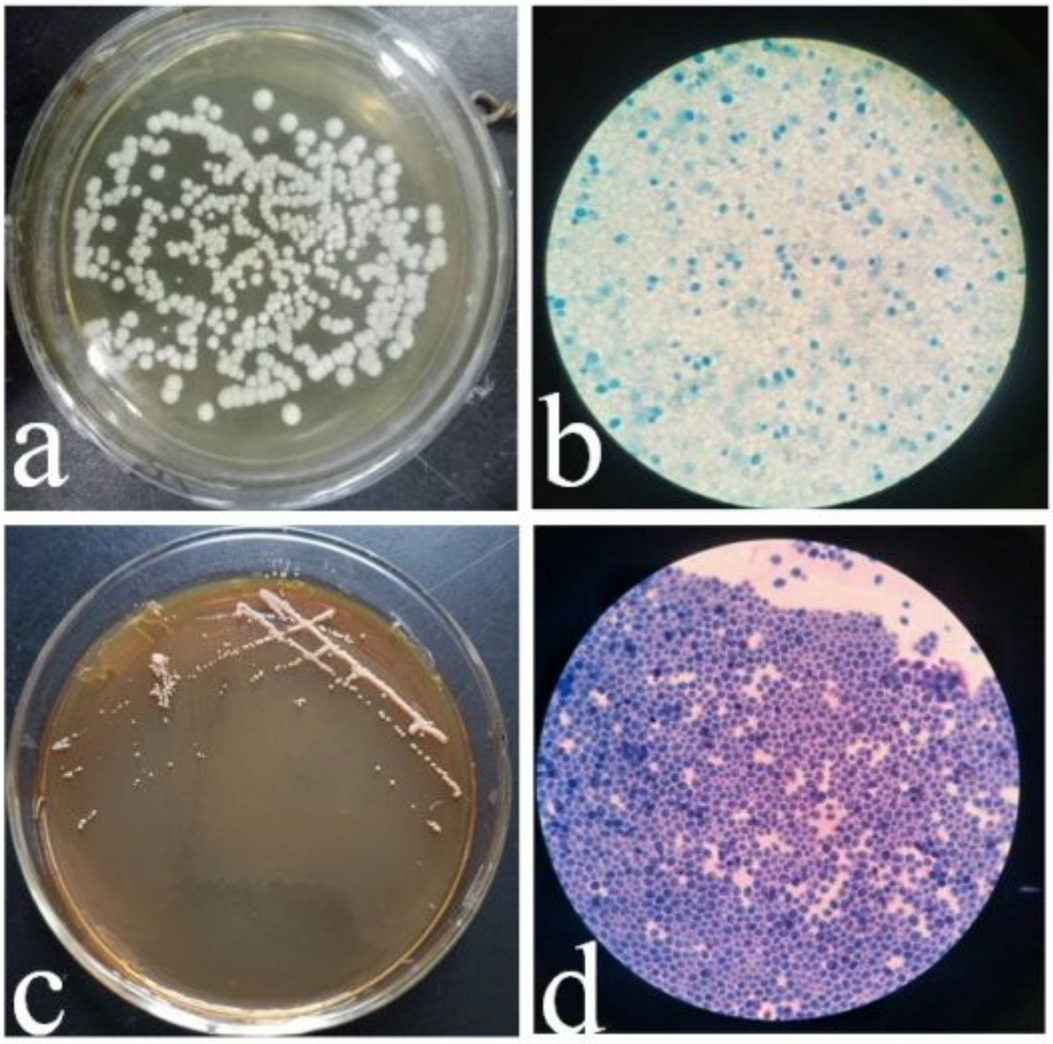

Figure 1

(a) Colony diagram at 28°C

(b) Microscopic examination diagram of Yeast at 28℃ (×100)

(c) Colony diagram at 43℃

(d) Microscopic examination diagram of Yeast at 43℃ (×100)

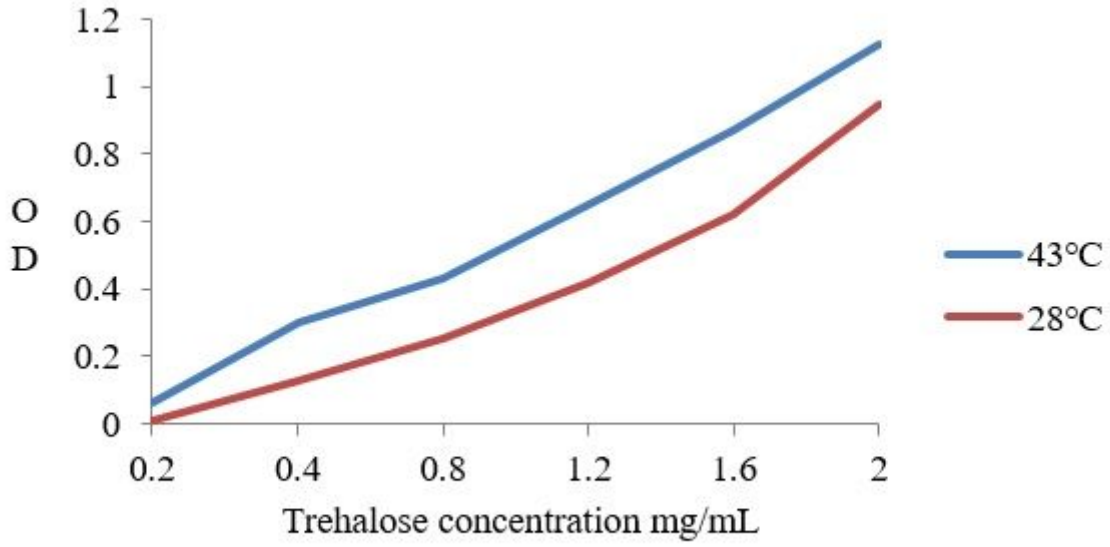

Figure 2

Standard curve of trehalose content measured by anthrone method with sulfuric acid

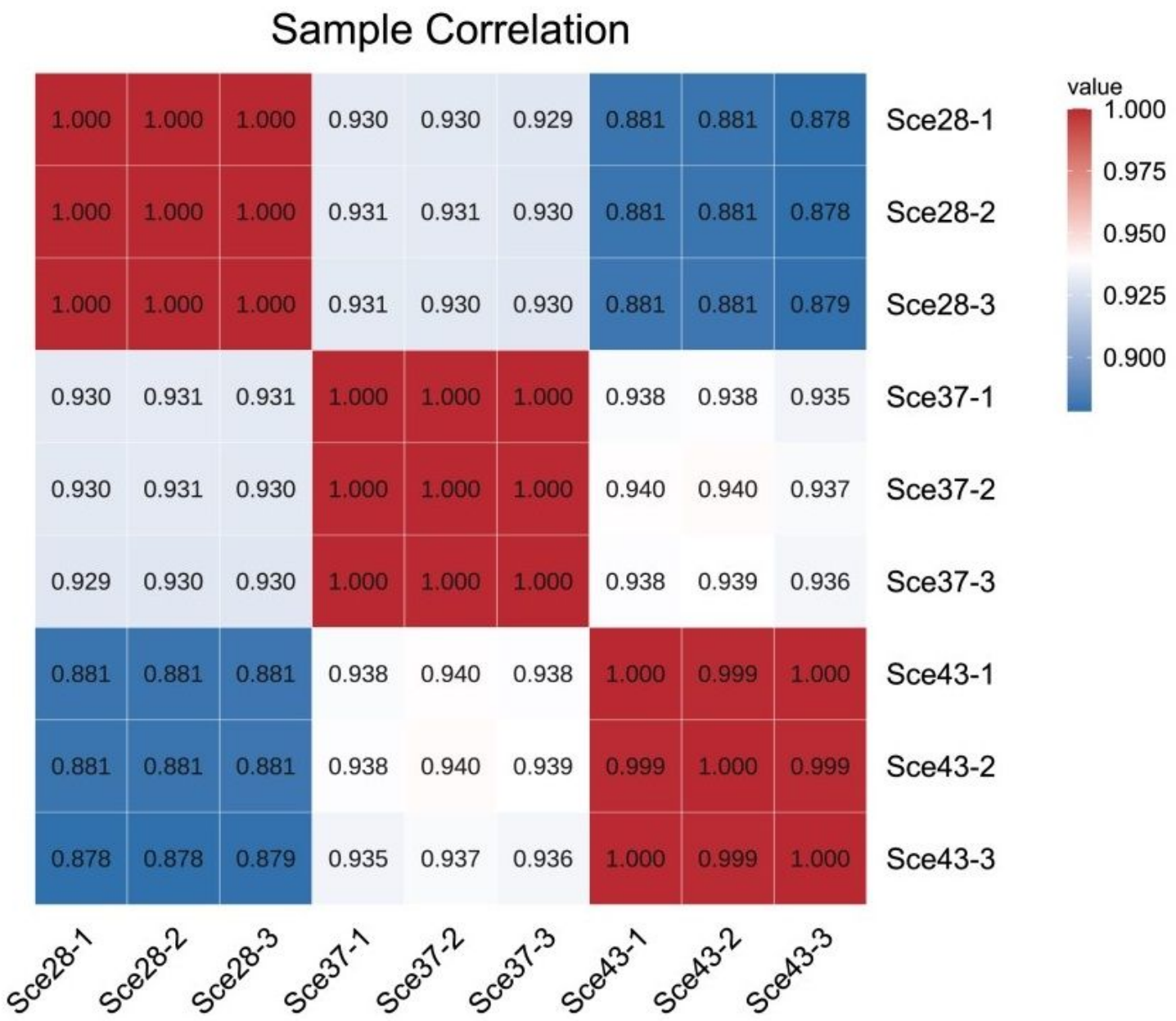

Figure 3

Heat map of correlation between samples

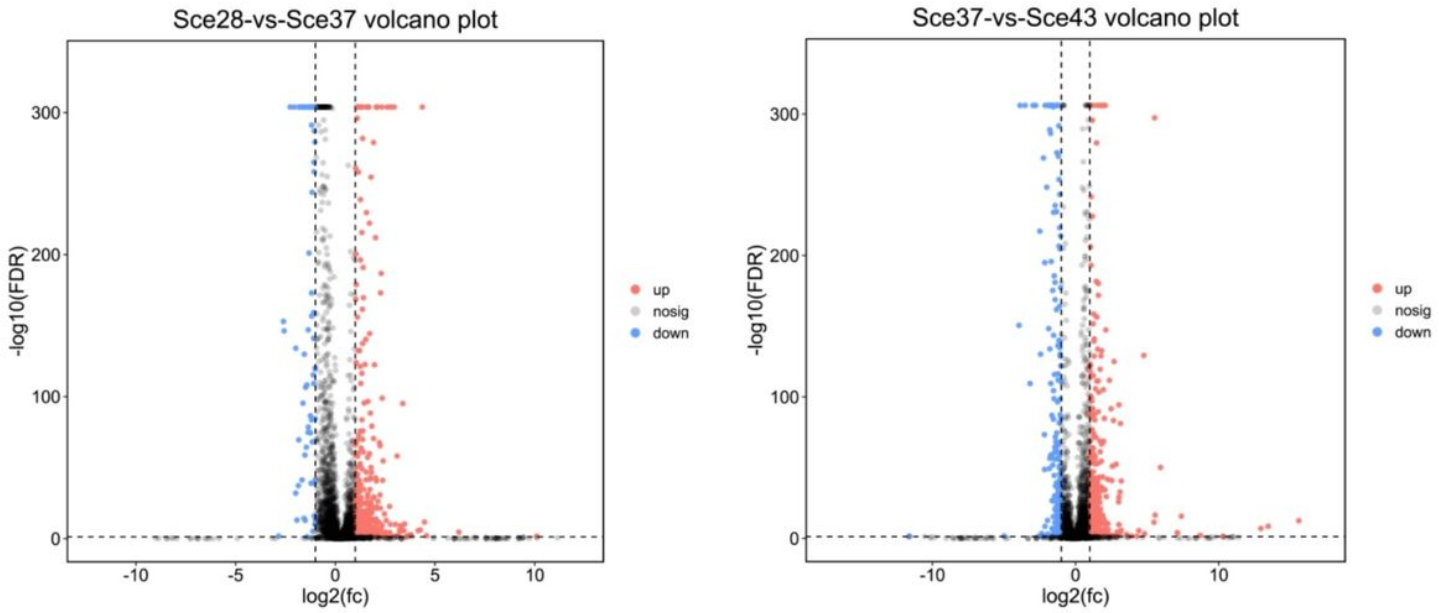

Figure 4

Volcano map with different expression levels

(left is the difference between 28 and 37 ºC, right is the difference between 37 and 43 ºC)

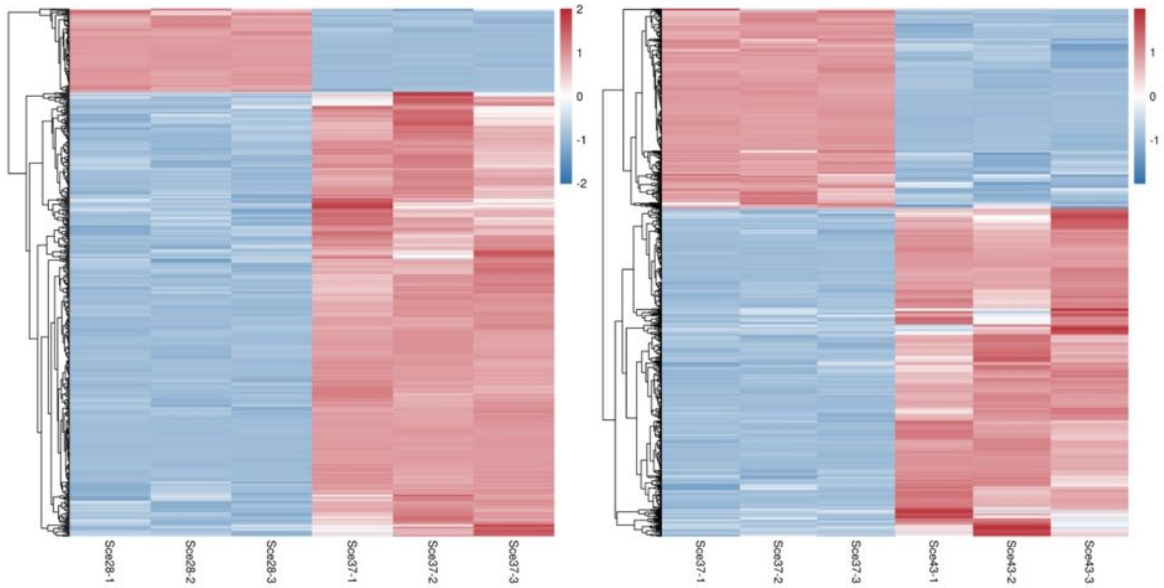

Figure 5

Heat map of gene clustering

(left: 28 and 37 ºC heat map, right: 37 and 43 ºC heat map)

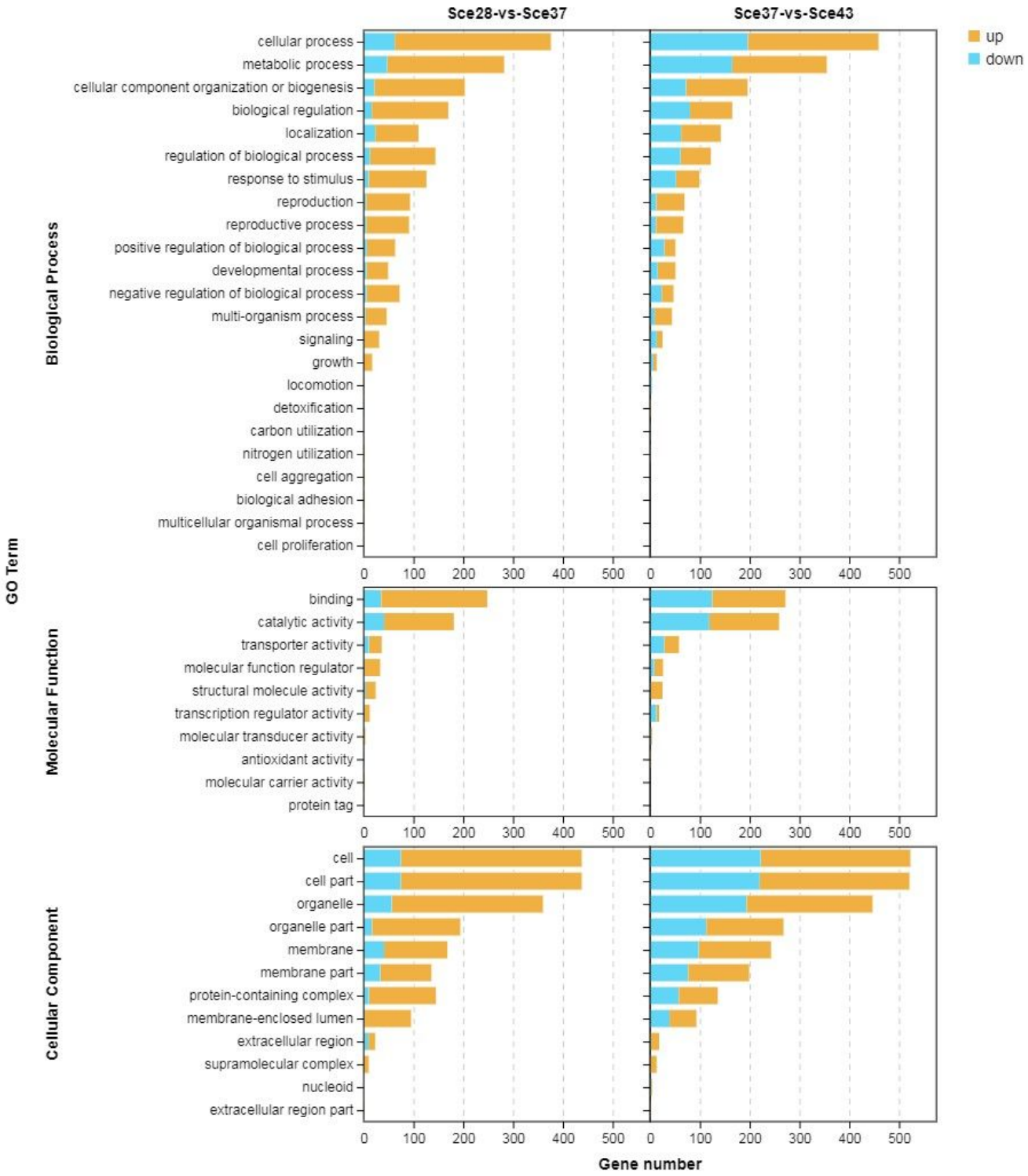

Figure 6

Statistical histogram of GO classification

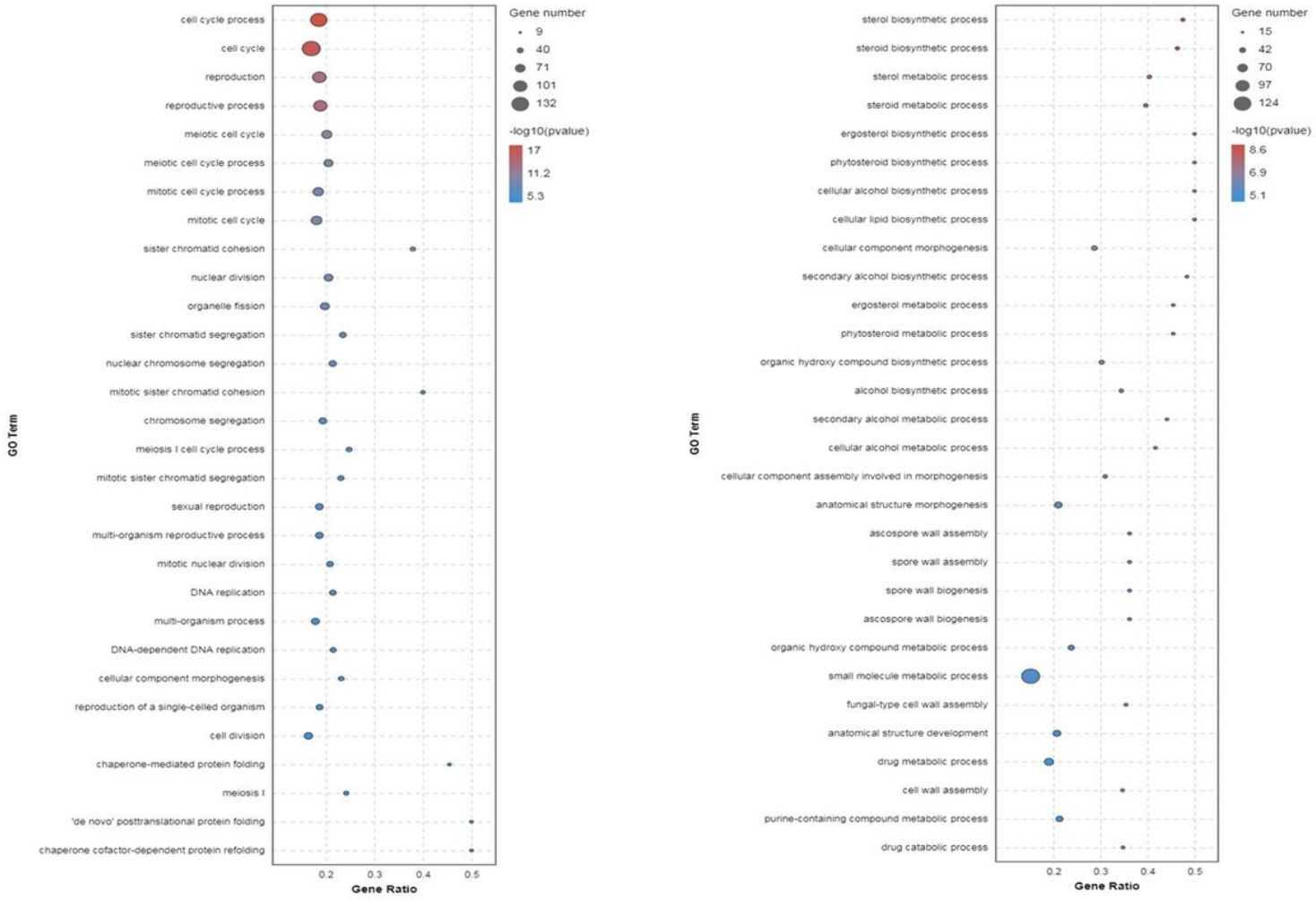

Figure 7

Bubble map of KEGG enrichment analysis：The enrichment bubble diagram at 28℃ and 37℃ is on the left, and the enrichment bubble diagram at 37℃ and 43℃ is on the right：