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A Transcriptomic Analysis of *Saccharomyces cerevisiae* Under the Stress of 2-Phenylethanol

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Abstract

2-Phenylethanol (2-PE) is a kind of advanced aromatic alcohol with rose fragrance, which is wildly used for the deployment of flavors and fragrances. Microbial transformation is the most feasible method for the production of natural 2-PE. But a bottleneck problem is the toxicity of 2-PE on the cells. The molecular mechanisms of the toxic effect of 2-PE to *Saccharomyces cerevisiae* are not well studied. In this study, we analyzed the transcriptomes of *S. cerevisiae* in the media with and without 2-PE, respectively, using Illumina RNA-Seq technology. We identified 580 differentially expressed genes between *S. cerevisiae* in two different treatments. GO and KEGG enrichment analyses of these genes suggested that most genes encoding mitochondrial proteins, cytoplasmic, and plasma membrane proteins were significantly up-regulated, whereas the enzymes related to amino acid metabolism were down-regulated. These results indicated that 2-PE suppressed the synthesis of plasma membrane proteins, which suppressed the transport of nutrients required for growth. The findings in this study will provide insight into the inhibitory mechanism of 2-PE to yeast and other microbes.

Introduction

2-Phenylethanol (2-PE) is a higher aromatic alcohol with a rose-like flavor and is becoming one of the most important fragrance chemicals in food, perfume, cosmetic, and other chemical industry [30]. Consumers prefer natural 2-PE since its chemically produced counterpart contains some harmful synthetic precursor and byproducts [7]. 2-PE occurs naturally in the essential oils of many flowers and plants, such as hyacinths, jasmine, rose, and lilies. Nevertheless, extraction from essential oils is costly, thus other sources such as microbial fermentation are found for natural 2-PE synthesis [5, 31]. An efficient approach to the biosynthesis of 2-PE is producing from the catabolism of L-phenylalanine (L-phe) via Ehrlich pathway in yeasts such as *Pichia fermentans, Saccharomyces cerevisiae, Hansenula anomala*, and *Kloeckera saturnus* [1, 5].

In *S. cerevisiae*, L-phe is transaminated to phenylpyruvate by transaminase Aro9, decarboxylated to phenylacetaldehyde by decarboxylase Aro10, and subsequently reduced to 2-PE by dehydrogenases [10]. *ARO9* and *ARO10* are not expressed in cultures with abundant nitrogen source, while they were induced to produce aromatic amino acid aminotransferase II during growth on L-phe as the sole nitrogen source [3]. There are six genes involved in dehydrogenation including five alcohol dehydrogenases genes *ADH1–5* and one formaldehyde dehydrogenase gene *SFA1*. Expression of any gene can catalyze the last step of Ehrlich pathway to generate 2-PE. Nevertheless, the cell growth is found to be completely inhibited in 4.0 g/L exogenous 2-PE because of its cytotoxicity [34]. The formation of even higher 2-PE concentrations is prevented by the toxic effect that it has on microorganisms. Studies have shown that 2-PE can increase the fluidity of cell membrane, reduce uptake of amino acids and glucose, and induce a respiratory deficiency to inhibit the growth of *S. cerevisiae* [35]. Liu et al. [20] investigated the *Penicillium italicum* gene expression changes in a 2-PE treatment comparing with control type. They found 1304 genes with

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significantly differential expression, which played important roles in a number of pathways including amino acids and protein biosynthesis, cell cycle, and cell death. However, to our knowledge, few experimental data about the effect of 2-PE on the gene expression in *S. cerevisiae* have been published so far.

This study aimed to identify the candidate factors involved in the regulation of cell responses to 2-PE at the transcriptional level. Therefore, we analyzed the high throughput-based transcriptome-wide mRNA expression profiles of *S. cerevisiae* after exposure to 2-PE. The function of some differentially expressed genes was analyzed.

Materials and Methods

Media and Tolerance Test

Strain of *S. cerevisiae* SH003 was isolated from the pit mud in distilleries. The strain was grown in seed medium (SM, glucose 30 g/L, peptone 20 g/L, yeast extract 10 g/L) and preserved in malt extract medium (MEM, peptone 4 g/L, glucose 10 g/L, yeast extract 3 g/L, malt extract 10 g/L). For the tolerance test, different concentrations of 2-PE (1–5 g/L) were added to SM medium, and plate dilution method was employed to determine the quantity of yeast cells. The *S. cerevisiae* cells were diluted by 1:100 using sterile water after being cultured in SM for 12 h. Then, 0.1 mL dilutions were sucked and spread to plates with different concentrations of 2-PE. The colony-forming units (CFU) were counted after culturing at 30 °C for 48 h. *S. cerevisiae* SH003 in experimental and control groups for RNA-Seq were cultivated in SM with and without 4 g/L 2-PE, respectively, at 30 °C with shaking at 200 rpm for 24 h prior to RNA isolation. Three biological replicate samples were carried out in each group.

RNA Isolation, Quantification, and Qualification

The yeast cells were harvested after 24 h of culture. Total RNA was extracted using the InviTrap Spin Universal RNA Mini kit (Stratec Molecular, Berlin, Germany) following the manufacturer's instructions. RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (Implen, CA, USA). RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). All of the samples were of the highest quality and had RIN values of 10.

RNA-Seq Profiling

RNA-Seq profiling was performed by Beijing Novogene Bioinformatics Technology Co., Ltd (Beijing, China). Briefly, fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5×). The first and second strand cDNAs were synthesized using random hexamer primer and reverse transcriptase. The fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA) to select the ones of preferentially 150–200 bp in length. Then 3 µL USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37 °C for 15 min followed by 5 min at 95 °C before PCR. The required fragments were enriched by PCR amplification and products were purified (AMPure XP system) and assessed. Finally, the libraries were sequenced on an Illumina HiSeq 2500 platform and 150 bp paired-end reads were generated.

Data Analysis

Clean reads were obtained by removing reads containing adapter, reads containing ploy-N and low quality reads from raw data. All pair-end clean reads were then aligned to reference sequences of *S. cerevisiae* (ftp://ftp.ensemblgenomes.org/pub/fungi/release-34/fasta/saccharomyces_cerevisiae/) using TopHat v2.0.9 [18] with at most two mismatches. The mapped reads of each sample were assembled by Cufflinks (v2.1.1) in a reference-based approach. It was run with 'min-frags-per-transfrag = 0' and '--library-type,' other parameters were set as default. The gene expression levels were calculated as FPKM (fragments per kilo-base of exon per million fragments mapped). Alternative splicing (AS) events were classified into 12 basic types by the software ASprofile v1.0 [8]. The number of AS events in each sample was estimated, separately. Differentially expressed genes (DEGs) were identified using a model based on the negative binomial distribution. Genes with a P adjust <0.05 were assigned as differentially expressed for biological replicates. All the DEGs were mapped to gene ontology terms in the database (GO, http://www.geneontology.org/) for functional annotation and to Kyoto Encyclopedia of Genes and Genomes database (KEGG, http://www.genome.jp/kegg/) for enrichment analysis.

Quantitative RT-PCR (qRT-PCR) Verification

Eight genes relative to mitochondrial membrane and 2-PE synthesis were selected for confirmation by qRT-PCR with SYBR Premix Ex Taq™ (TakaRa, Japan). Primers were designed with Primer Premier 5.0 and synthesized

by Sangon Biotech Co., Ltd. (Shanghai, China) (Supplemental Table S3). PCR amplification was conducted in a 10 µL reaction system containing 5 µL SYBR® Premix Ex Taq™ (Tli RNase H Plus) (TaKaRa, Japan) and performed on a QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, USA). The cycling conditions were 95 °C for 30 s followed by 45 cycling of 95 °C for 5 s, 60 °C for 30 s. Three biological replicates were performed for each target gene. The relative expression levels of DEGs were analyzed using the $2^{-\Delta\Delta C_T}$ method and normalized to the reference genes β-tubulin.

Results

Cell Growth in Media with 2-PE

The number of *S. cerevisiae* SH003 colonies on SM medium plates containing different concentrations of 2-PE is shown in Table 1. The CFU in SM plant with 0 g/L 2-PE could be regarded as the concentration of cells at time zero of treatment. Thus it can be seen that the cells concentration decreased under the stress of 2-PE relatively to time zero of treatment. Moreover, the colony-forming units decreased with the increase of 2-PE concentration. When the concentration reached to 3.5 g/L, the number of colonies was considerably decreased, and no colonies grew on the plate with 5 g/L 2-PE.

RNA-Seq Analysis

We sequenced the transcriptome of *S. cerevisiae* SH003 cultured in SM containing 4 g/L (PES) and 0 g/L (control, CK) 2-PE, respectively, for 24 h. Row reads of the six samples were submitted to the Sequence Read Archive (SRA) database of NCBI (SRR5451305). The paired-end reads of 150 bp in length obtained from each sample are shown in Table 2. We totally mapped 77046369 (85.81%), 76228930 (85.93%), 70396112 (81.31%), 76550118 (86.72%), 111365787 (86.58%), 70262097 (85.09%) reads to the genome of *S. cerevisiae*. The number of uniquely mapped reads in PES was fewer than that in CK.

Alternative splicing is classified into 12 types that are TSS (Alternative 5′ first exon), TTS (Alternative 3′ last exon), SKIP (Skipped exon), XSKIP (Approximate SKIP), MSKIP (Multi-exon SKIP), XMSKIP (Approximate MSKIP), IR (Intron retention), XIR (Approximate IR), MIR (Multi-IR), XMIR (Approximate MIR), AE (Alternative exon ends), XAE (Approximate AE), respectively. As shown in Fig. 1, the TSS and TTS were the most in each sample, which in PES were less than CK. XSKIP and SKIP existed in CK but not in PES.

With the parameter $|\log2(\text{fold change})| \geq 2$ and $q \leq 0.01$, 580 DEGs were detected in the condition of 2-PE. Among them, 419 genes were up-regulated and the others were down-regulated. This indicated that the growth of *S. cerevisiae* was affected by 2-PE and some emergency mechanisms were activated for environmental stress. There were 13 genes with known function among the top 20 DEGs, which encoded hydrophilin, plasma membrane H+-ATPase, succinate dehydrogenase, catalase, ATPase, membrane protein, and oligopeptide transporter (Table 3). Most of them were reported to be involved in the component and function of membranes like the component of the TOM (translocase of the outer membrane) complex, which was the most significantly up-regulated gene. TOM is a complex of proteins in

Table 1 Colony-forming units of *S. cerevisiae* SH003 in plates containing different concentrations of 2-PE

Concentration of 2-PE (g/L)	0	1	2	3	3.5	4	5
Colony-forming units (CFU/mL[a])	73	68	64	62	41	19	0

[a]CFU/mL means colony-forming units per mL sample

Table 2 Summary of mapping results

	Samples					
	CK_1	CK_2	CK_3	PES_1	PES_2	PES_3
Total reads	88274244	128621652	82569928	89789064	88712088	86581560
Reads mapped to genome	76550118 (86.72%)	111365787 (86.58%)	70262097 (85.09%)	77046369 (85.81%)	76228930 (85.93%)	70396112 (81.31%)
Multiple mapped reads	919515 (1.04%)	1403646 (1.09%)	862905 (1.05%)	1259549 (1.4%)	1213139 (1.37%)	1441414 (1.66%)
Uniquely mapped reads	75630603 (85.68%)	109962141 (85.49%)	69399192 (84.05%)	75786820 (84.41%)	75015791 (84.56%)	68954698 (79.64%)

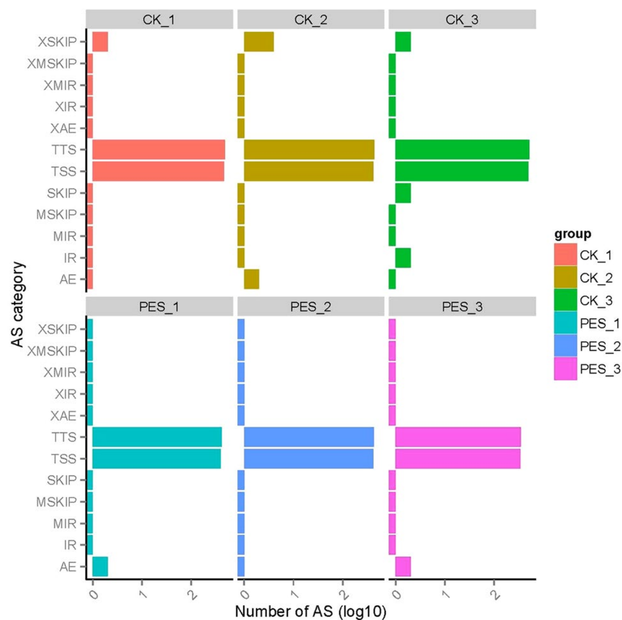

Fig. 1 The statistics of AS events. XSKIP, Approximate SKIP (XSKIP_ON, XSKIP_OFF pair); XMSKIP, Approximate MSKIP (XMSKIP_ON, XMSKIP_OFF pair); XMIR, Approximate MIR (XMIR_ON, XMIR_OFF pair); XIR, Approximate IR (XIR_ON, XIR_OFF pair); XAE, Approximate AE; TTS, Alternative 3′ last exon (transcription terminal site); TSS, Alternative 5′ first exon (transcription start site); SKIP, Skipped exon (SKIP_ON, SKIP_OFF pair); MSKIP, Multi-exon SKIP (MSKIP_ON, MSKIP_OFF pair); MIR, Multi-IR (MIR_ON, MIR_OFF pair); IR, Intron retention (IR_ON, IR_OFF pair); AE, Alternative exon ends (5′, 3′, or both). (Color figure online)

the outer mitochondrial membrane, which can guide proteins entering into the intermembrane space of the mitochondrion through the outer membrane [19]. The high expression level of TOM indicated that mitochondrion was quite active and more energy was needed for cell growth and metabolism.

GO enrichment analysis of DEGs (Fig. 2) showed that nineteen gene categories were defined on the basis of cellular component. Major categories were cytoplasm, cytoplasmic part, mitochondrion, and membrane. Metabolic process was significantly enriched in the biological process category. The genes were classified on the basis of molecular function into cofactor binding and oxidoreductase activity.

The PEA-responsive genes were further assessed using KEGG pathway analysis. A total of 20 different metabolic pathways were found with more than 12 affiliated genes (Data were listed in Supplemental Table S1), of which some were consistent with biological processes that were already identified by GO analysis. As shown in Fig. 3, the prominent related pathways were carbon metabolism, protein processing in endoplasmic reticulum, glycolysis, peroxisome, fatty acid degradation, amino acid biosynthesis and metabolism, and sphingolipid metabolism. Most genes were relative to metabolic pathways. Data indicated that cytoplasm and membrane were the major reactive locations in response to 2-PE, which is in accord with GO enrichment data.

qTR-PCR Verification

To confirm that the DEGs identified by deep sequencing were indeed differentially expressed, a total of eight genes were chosen for confirmation in a biologically independent experiment using qRT-PCR, including proteasome, membrane protein, and glycometabolism-related genes, which were detected in the transcriptome and bioinformatics analyses. qRT-PCR analysis showed that the expression changes of the chosen genes were in agreement with those showed by RNA-Seq (Fig. 4), although there were some differences in the degree of the changes. It indicated that the RNA-Seq results were convincible.

Discussion

The repression of 2-PE on the growth and metabolism of strains is the bottleneck of industrial production by microorganism. In this study, we investigated the expression profiles of *S. cerevisiae* under the stress of 2-PE by RNA-Seq. The differences in gene splicing suggested that 2-PE caused differences in the gene expression. Through functional enrichment analysis, we identified 580 DEGs (Supplemental Table S2) of *S. cerevisiae* which are involved in mitochondrial activity, plasma membrane permeability, amino acid metabolism, meiosis, and so on.

Mitochondrial Proteins

Mitochondrion is a double membrane-bound organelle containing its own DNA found in all eukaryotic organisms, though most mitochondrial proteins are translated in the cytoplasm and transported specifically into mitochondria [11]. Only eight proteins in yeast are encoded by the mitochondrial DNA and synthesized on mitochondrial ribosomes [27]. Most proteins are transported into different mitochondrial subcompartments from cytoplasm via several distinct translocases in the mitochondrial outer membrane (TOM complex) and inner membrane (TIM complex) [23]. The TOM complex of *S. cerevisiae*, which is regarded as a universal gateway for mitochondria, contains a channel-forming subunit (Tom40), receptors for various precursor proteins (Tom20 and Tom70), an internal receptor for mitochondrial precursor proteins which also preserves the complex structure (Tom22), and regulators of the complex assembly and stability (Tom5,

Table 3 List of the 20 most up- or down-regulated genes of *S. cerevisiae* in 2-PE

Gene id	Gene name	log2(fold change)	Description
YPL223C	GRE1	−9.08624	Hydrophilin essential in desiccation–rehydration process
YHR139C	SPS100	−8.38915	Protein required for spore wall maturation
YMR175W	SIP18	−8.37597	Phospholipid-binding hydrophilin
YPL036W	PMA2	−6.31423	Plasma membrane H+-ATPase
YLR164W	SHH4	−6.07089	Subunit of succinate dehydrogenase (SDH)
YKL187C	FAT3	−5.97522	Protein required for fatty acid uptake
YGR088W	CTT1	−5.84167	Cytosolic catalase T
YMR174C	PAI3	−5.76963	Cytoplasmic proteinase A (Pep4p) inhibitor
YMR118C	SHH3	−5.37293	Putative mitochondrial inner membrane protein
YHR096C	HXT5	−5.32316	Hexose transporter with moderate affinity for glucose, induced by a decrease in growth rate
YBL075C	SSA3	−5.13908	ATPase plays a role in SRP-dependent cotranslational protein membrane targeting and translocation
YGR236C	SPG1	−5.12331	Protein required for high temperature survival during stationary phase
YOL101C	IZH4	−4.99563	Membrane protein involved in zinc ion homeostasis
YDL222C	FMP45	−4.94491	Integral membrane protein localized to mitochondria
YPR133W-A	TOM5	Inf[a]	Component of the TOM (translocase of outer membrane) complex
YBR056W-A		7.05289	
YJL133C-A		5.47543	
YPR194C	OPT2	5.32807	Oligopeptide transporter
YBR056C-B		5.1474	
YPL251W		4.90211	

[a]Inf means infinite, whose expression level was 0 in CK, while the expression in 2-PE treatment was very high (RPFM = 21749)

Tom6, and Tom7) [33]. It is in charge of the translocation of entered proteins across or into the outer membrane and deciphers their targeting signals and subsequent sorting. The TIM complexes include two distinct translocases: the TIM23 complex and the TIM22 complex. Precursors with an N-terminal presequence are imported via the TIM23 complex, whereas mitochondrial carrier proteins like the ADP/ATP carrier that are synthesized without a presequence require the TIM22 complex for insertion into the inner membrane [2]. Nearly all mitoribosomal proteins and translational factors are encoded by nuclear DNA and imported from the cytoplasm via TOM and TIM complexes [6].

Our study showed that several genes of mitochondrial proteins such as mitoribosomal large and small subunits, TOM proteins, both TIM22 and TIM23 complexes were up-regulated under the stress of 2-PE. The up-regulated expression of these proteins indicated that the activity of mitochondria in *S. cerevisiae* was increased. Mitochondria play a critical role in cellular bioenergetics by virtue of ATP generation in the cell. They will accelerate division and enhance activity under stressful condition to increase ATP production for normal cell physiology [28]. This is to say, 2-PE caused non-specific cellular damage to *S. cerevisiae*.

Plasma Membrane Proteins

The plasma membrane forms a barrier for hydrophilic molecules such as amino acids, sugars, and ions. Specialized proteins mediate the selective uptake and/or secretion of solutes across this membrane [36]. Ammonium permeases, encoded by *MEP* genes, are proteins located in the cell membrane that transport NH_4^+ from medium to increase the intracellular concentration of ammonia for growth [21]. Marini et al. [22] found that the *MEP* genes were most highly expressed when the cells grew on low concentrations of ammonium or on ‘poor’ nitrogen sources. In our study, ammonium permease gene (ID: YGR121C) was up-regulated significantly, which indicated that 2-PE inhibited the uptake of nitrogen. Besides, channels for Ca^{2+} and other ions were also up-regulated. *S. cerevisiae* cells use Ca^{2+} as a second messenger when they are exposed to various environmental stress conditions [26]. The increase of cytosolic Ca^{2+} can be achieved from external Ca^{2+} by the Ca^{2+} channel in plasma membrane. Many other anions and cations, such as K^+, Na^+, Cl^-, also penetrate the plasma membrane through specific channels. These channels play important roles in osmoregulation and the membrane potential balance.

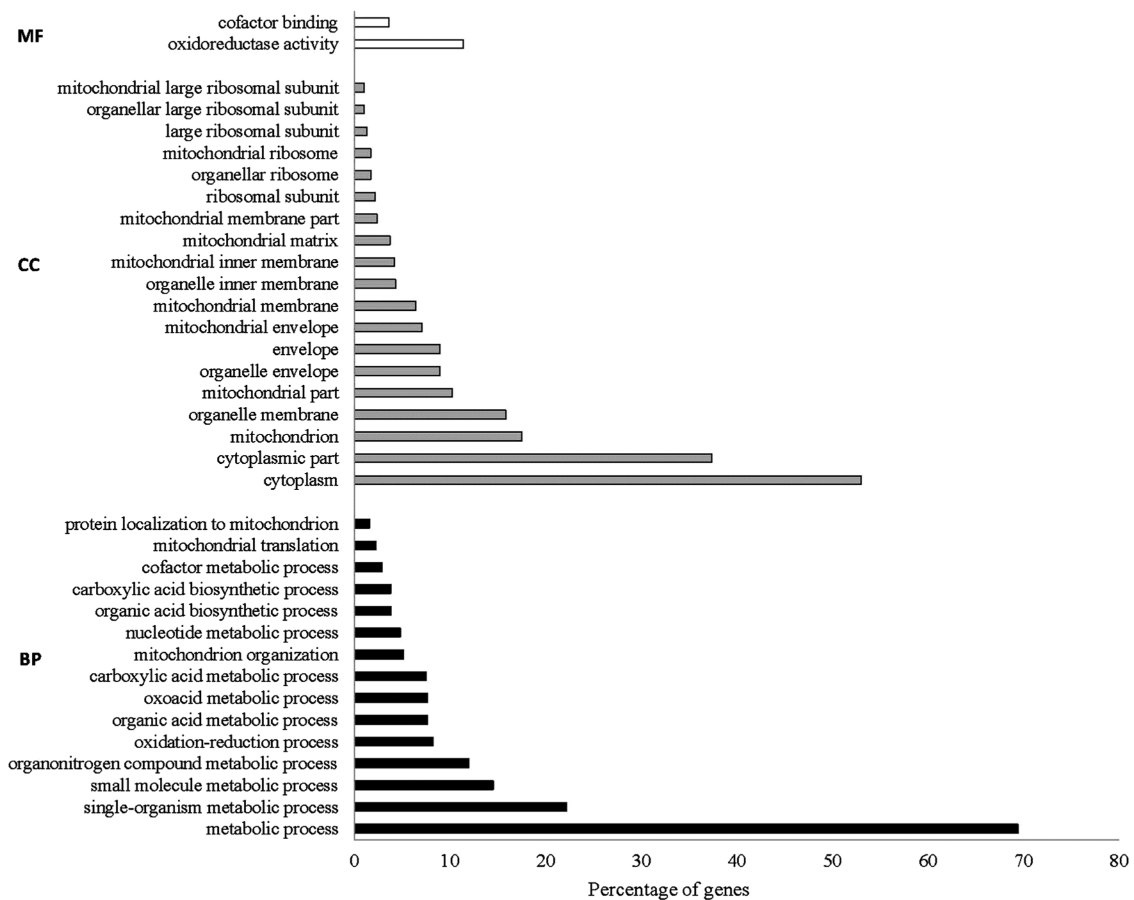

Fig. 2 The most enriched GO terms. Bar chart of DEGs enriched in GO term; it can directly reflect the percentage of DEGs distributing into different GO terms. *CC* cellular component, *MF* molecular function, *BP* biological process

Aminotransferase, Decarboxylase, and Dehydrogenase

In *S. cerevisiae*, bioconversion of L-phe to 2-PE is completed via the Ehrlich pathway: L-phe is transaminated to phenylpyruvate by aminotransferase and then decarboxylated to phenylacetaldehyde by phenylpyruvate decarboxylase, followed by reduction to 2-PE by alcohol dehydrogenase. Aromatic aminotransferase II (Aro9) and phenylpyruvate decarboxylase (Aro10), which are highly induced by aromatic amino acids and play important roles in aromatic amino acid catabolism [9], were high expressed in the presence of 2-PE. Nevertheless, the alcohol dehydrogenase (Adh) genes were down-regulated. This result indicated that high concentration of 2-PE play feedback repression in the synthesis of alcohol dehydrogenase. The inhibition of this enzyme resulted in the limitation of 2-PE production [17]. Cat8 is a zinc cluster protein as well as a transcription factor, which is a regulator for derepression of alcohol dehydrogenase gene of the yeast *S. cerevisiae* [37]. Wang et al. [38] reported that Cat8 could activate the transcription of phenylpyruvate decarboxylase gene *ARO9* and *ARO10*. Moreover, both Aro80 and Mig1 are zinc finger transcriptional factors. Aro80 can activate transcription of *ARO9* and *ARO10*, while Mig1 represses the transcription of these genes [15, 29]. In our results, the expression of *CAT8* in experimental group had no obvious change. *ARO80* and *MIG1* were slightly up- and down-regulated, respectively. Based on the above results and our previous investigation, we put forward the hypothesis that 2-PE may affect the expression of some transcription factors and further regulate the transcription of *ARO9, ARO10*, and *ADH* genes.

Proteins Involved in Meiosis and Sporulation

Cells of *S. cerevisiae* follow the mitotic cycle and proliferate by budding in the presence of adequate nutrient supply. When the nutrient elements are deficient, haploid cells enter a dormant stationary phase, whereas diploid cells initiate a differentiation pathway called sporulation [24]. Starvation for nitrogen, the absence of a fermentable

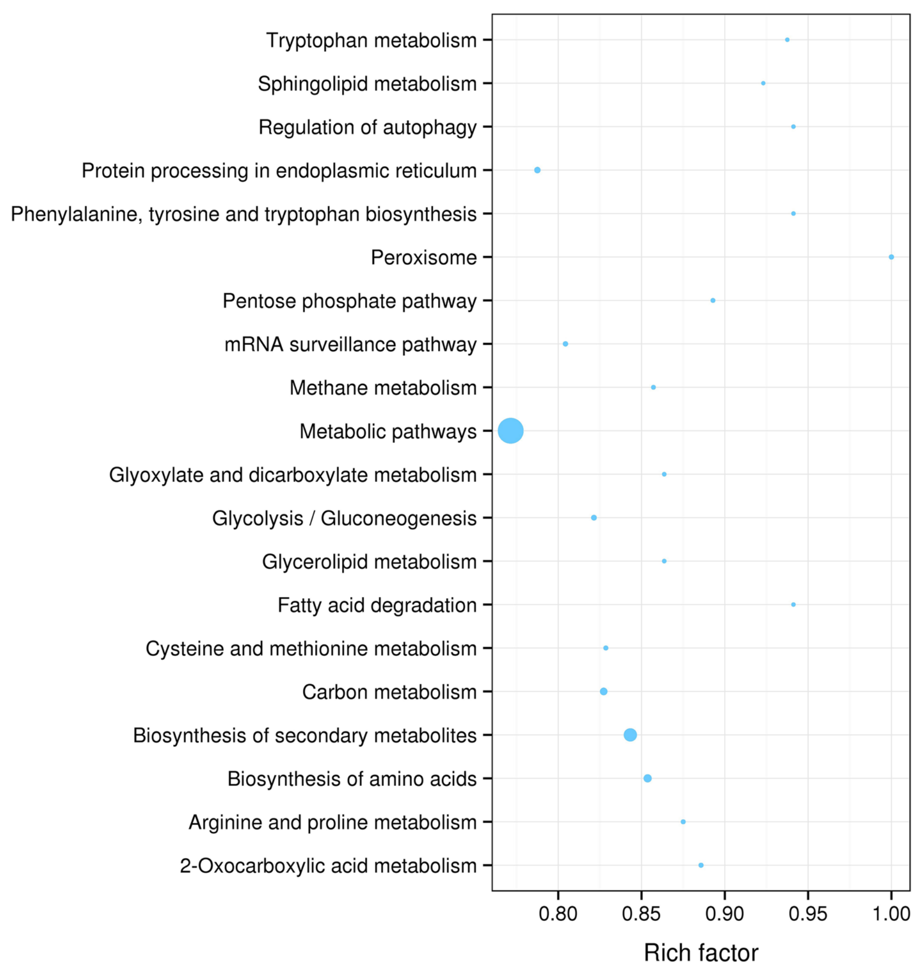

Fig. 3 The statistics of pathway enrichment of DEGs. The size of the dots indicates the number of DGEs in this pathway, and the greater the cross axis value means the higher enrichment

carbon source, and the presence of a non-fermentable carbon source controls the entry into sporulation [12, 13, 16].

The genetic and environmental signals activate a range of regulatory proteins that coordinate the expression of genes involved in various sporulation-specific processes. According to the time of induction, sporulation-specific genes were divided into three major sequential groups: early, middle, and late genes [24]. The set of early genes is induced by Ime1 and Ime2 (Inducer of Meiosis) [4, 32]. The major regulator of middle genes is Ndt80p [25]. A very small (67 amino acid) protein encoded by the gene *SPO24* is functionally important in mid-late meiosis [14], whereas the factors required for the activation of the late genes remain obscure.

It was found that the mRNAs of genes involved in meiotic recombination and spore wall formation were multiply increased in our study. This indicated that the yeast cells did not uptake enough nutrition, and spores were formed in the presence of 2-PE. However, some genes related to normal sporulation were down-regulated. Further studies were needed to explore these results.

Conclusions

In this paper, comparative transcriptome analysis of *S. cerevisiae* in the media containing 2-PE and the control was carried out by RNA-Seq. This paper is the first attempt to demonstrate the molecular mechanism induced in yeast under the stress of 2-PE. The results showed that genes encoding mitochondrial proteins, cytoplasmic, and plasma membrane proteins were significantly up-regulated, whereas the enzymes related to amino acid metabolism were down-regulated. We speculated that 2-PE suppressed the synthesis of plasma membrane proteins, which hindered the transport of nutrients required for growth. Conversely, cells enhanced the mitochondrial activity and formed spores to respond the stress of 2-PE.

Until now, lack of the knowledge of the inhibitory mechanism of 2-PE to *S. cerevisiae* makes the majority of the study on 2-PE biosynthesis with higher productivity focus on selection of species, traditional breeding of yeast strains, medium optimization, or in situ 2-PE removal. Our

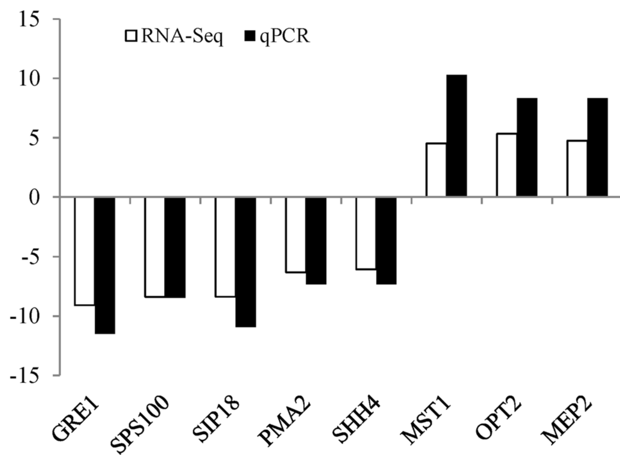

Fig. 4 Validation of DEGs by qRT-PCR analysis. The relative expression levels of eight differential expressed genes in *S. cerevisiae* in the condition of 2-PE were obtained by RNA-Seq (white) and by qRT-PCR (black). *GRE1* hydrophilin essential in desiccation–rehydration process, *SPS100* protein required for spore wall maturation, *SIP18* phospholipid-binding hydrophilin, *PMA2* plasma membrane H+-ATPase, *SHH4* subunit of succinate dehydrogenase, *MST1* mitochondrial threonyl-tRNA synthetase, *OPT2* oligopeptide transporter, *MEP2* ammonium permease. The y-axis stood for the relative expression levels [log2(foldchange)]

research provided a preliminary study on molecular mechanisms induced by 2-PE in *S. cerevisiae*, and further studies are required to determine the function of up-regulated genes. The findings will provide insight into the inhibitory mechanism of 2-PE and the development of molecular breeding for 2-PE industrial production.

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References

1. Albertazzi E, Cardillo R, Servi S, Zucchi G (1994) Biogeneration of 2-phenylethanol and 2-phenylethylacetate important aroma components. Biotechnol Lett 16:491–496
2. Bauer MF, Hofmann S, Neupert W, Brunner M (2000) Protein translocation into mitochondria: the role of TIM complexes. Trends Cell Biol 10:25–31
3. Boer VM, Tai SL, Vuralhan Z, Arifin Y, Walsh MC, Piper MD, de Winde JH, Pronk JT, Daran JM (2007) Transcriptional responses of *Saccharomyces cerevisiae* to preferred and nonpreferred nitrogen sources in glucose-limited chemostat cultures. FEMS Yeast Res 7:604–620
4. Brush GS, Najor NA, Dombkowski AA, Cukovic D, Sawarynski KE (2012) Yeast IME2 functions early in meiosis upstream of cell cycle-regulated SBF and MBF targets. PLoS ONE 7:e31575
5. Chung HJ, Lee SL, Chou CC (2000) Production and molar yield of 2-phenylethanol by *Pichia fermentans* L-5 as affected by some medium components. J Biosci Bioeng 90:142–147
6. Desai N, Brown A, Amunts A, Ramakrishnan V (2017) The structure of the yeast mitochondrial ribosome. Science 355:528–531
7. Eshkol N, Sendovski M, Bahalul M, Katz-Ezov T, Kashi Y, Fishman A (2009) Production of 2-phenylethanol from L-phenylalanine by a stress tolerant *Saccharomyces cerevisiae* strain. J Appl Microbiol 106:534–542
8. Florea L, Song L, Salzberg SL (2013) Thousands of exon skipping events differentiate among splicing patterns in sixteen human tissues. F1000Research 2:188
9. Godard P, Urrestarazu A, Vissers S, Kontos K, Bontempi G, van Helden J, Andre B (2007) Effect of 21 different nitrogen sources on global gene expression in the yeast *Saccharomyces cerevisiae*. Mol Cell Biol 27:3065–3086
10. Hazelwood LA, Daran JM, van Maris AJ, Pronk JT, Dickinson JR (2008) The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. Appl Environ Microbiol 74:2259–2266
11. Herrmann JM, Woellhaf MW, Bonnefoy N (2013) Control of protein synthesis in yeast mitochondria: the concept of translational activators. Biochim Biophys Acta 1833:286–294
12. Hirschberg J, Simchen G (1977) Commitment to the mitotic cell cycle in yeast in relation to meiosis. Exp Cell Res 105:245–252
13. Honigberg SM, Purnapatre K (2003) Signal pathway integration in the switch from the mitotic cell cycle to meiosis in yeast. J Cell Sci 11:2137–2147
14. Hurtado S, Kim GK, Sontheimer EJ (2014) SPO24 is a transcriptionally dynamic, small ORF-encoding locus required for efficient sporulation in *Saccharomyces cerevisiae*. PLoS ONE 9:e105058
15. Iraqui I, Vissers S, Andre B, Urrestarazu A (1999) Transcriptional induction by aromatic amino acids in *Saccharomyces cerevisiae*. Mol Cell Biol 19:3360–3371
16. Jambhekar A, Amon A (2008) Control of meiosis by respiration. Curr Biol 18:969–975
17. Kim B, Cho BR, Hahn JS (2014) Metabolic engineering of *Saccharomyces cerevisiae* for the production of 2-phenylethanol via Ehrlich pathway. Biotechnol Bioeng 111:115–124
18. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 14:R36
19. Kuszak AJ, Jacobs D, Gurnev PA, Shiota T, Louis JM, Lithgow T, Bezrukov SM, Rostovtseva TK, Buchanan SK (2015) Evidence of distinct channel conformations and substrate binding affinities for the mitochondrial outer membrane protein translocase pore Tom40. J Biol Chem 290:26204–26217
20. Liu P, Cheng Y, Yang M, Liu Y, Chen K, Long CA, Deng X (2014) Mechanisms of action for 2-phenylethanol isolated from *Kloeckera apiculata* in control of *Penicillium* molds of citrus fruits. BMC Microbiol 14:242
21. Magasanik B (2003) Ammonia assimilation by *Saccharomyces cerevisiae*. Eukaryot Cell 2:827–829
22. Marini AM, Vissers S, Urrestarazu A, Andre B (1994) Cloning and expression of the MEP1 gene encoding an ammonium transporter in *Saccharomyces cerevisiae*. EMBO J 13:3456–3463
23. Neupert W (1997) Protein import into mitochondria. Annu Rev Biochem 66:863–917
24. Piekarska I, Rytka J, Rempola B (2010) Regulation of sporulation in the yeast *Saccharomyces cerevisiae*. Acta Biochim Pol 57:241–250
25. Pierce M, Benjamin KR, Montano SP, Georgiadis MM, Winter E, Vershon AK (2003) Sum1 and Ndt80 proteins compete for binding to middle sporulation element sequences that control meiotic gene expression. Mol Cell Biol 23:4814–4825
26. Popa CV, Dumitru I, Ruta LL, Danet AF, Farcasanu IC (2010) Exogenous oxidative stress induces Ca^{2+} release in the yeast *Saccharomyces cerevisiae*. FEBS J 277:4027–4038

27. Reichert AS, Neupert W (2002) Contact sites between the outer and inner membrane of mitochondria-role in protein transport. Biochim Biophys Acta 1592:41–49
28. Roy M, Reddy PH, Iijima M, Sesaki H (2015) Mitochondrial division and fusion in metabolism. Curr Opin Cell Biol 33:111–118
29. Schuller HJ (2003) Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae*. Curr Genet 43:139–160
30. Sendovski M, Nir N, Fishman A (2010) Bioproduction of 2-phenylethanol in a biphasic ionic liquid aqueous system. J Agric Food Chem 58:2260–2265
31. Serp D, von Stockar U, Marison IW (2003) Enhancement of 2-phenylethanol productivity by *Saccharomyces cerevisiae* in two-phase fed-batch fermentations using solvent immobilization. Biotechnol Bioeng 82:103–110
32. Smith HE, Driscoll SE, Sia RA, Yuan HE, Mitchell AP (1993) Genetic evidence for transcriptional activation by the yeast IME1 gene product. Genetics 133:775–784
33. Sokol AM, Sztolsztener ME, Wasilewski M, Heinz E, Chacinska A (2014) Mitochondrial protein translocases for survival and well-being. FEBS Lett 588:2484–2495
34. Stark D, Munch T, Sonnleitner B, Marison IW, von Stockar U (2002) Extractive bioconversion of 2-phenylethanol from L-phenylalanine by *Saccharomyces cerevisiae*. Biotechnol Prog 18:514–523
35. Stark D, Zala D, Münch T, Sonnleitner B, Marison IW, Stockar U (2003) Inhibition aspects of the bioconversion of L-phenylalanine to 2-phenylethanol by *Saccharomyces cerevisiae*. Enzyme Microb Technol 32:113–212
36. Van der Rest ME, Kamminga AH, Nakano A, Anraku Y, Poolman B, Konings WN (1995) The plasma membrane of *Saccharomyces cerevisiae*: structure, function, and biogenesis. Microbiol Rev 59:304–322
37. Walther K, Schuller HJ (2001) Adr1 and Cat8 synergistically activate the glucose-regulated alcohol dehydrogenase gene ADH2 of the yeast *Saccharomyces cerevisiae*. Microbiology 147:2037–2044
38. Wang Z, Bai X, Guo X, He X (2017) Regulation of crucial enzymes and transcription factors on 2-phenylethanol biosynthesis via Ehrlich pathway in *Saccharomyces cerevisiae*. J Ind Microbiol Biotechnol 44:129–139