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RESEARCH

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Exploring the stress response mechanisms to 2-phenylethanol conferred by Pdr1p mutation in *Saccharomyces cerevisiae*

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Abstract

Background The 2-phenylethanol (2-PE) tolerance phenotype is crucial to the production of 2-PE, and Pdr1p mutation can significantly increase the tolerance of 2-PE in *Saccharomyces cerevisiae*. However, its underlying molecular mechanisms are still unclear, hindering the rational design of superior 2-PE tolerance performance.

Results Here, the physiology and biochemistry of the *PDR1*_862 and 5D strains were analyzed. At 3.5 g/L 2-PE, the ethanol concentration of *PDR1*_862 decreased by 21%, and the 2-PE production of *PDR1*_862 increased by 16% than those of 5D strain. Transcriptome analysis showed that at 2-PE stress, Pdr1p mutation increased the expression of genes involved in the Ehrlich pathway. In addition, Pdr1p mutation attenuated sulfur metabolism and enhanced the one-carbon pool by folate to resist 2-PE stress. These metabolic pathways were closely associated with amino acids metabolism. Furthermore, at 3.5 g/L 2-PE, the free amino acids content of *PDR1*_862 decreased by 31% than that of 5D strain, among the free amino acids, cysteine was key amino acid for the enhancement of 2-PE stress tolerance conferred by Pdr1p mutation.

Conclusions The above results indicated that Pdr1p mutation enhanced the Ehrlich pathway to improve 2-PE production of *S. cerevisiae*, and Pdr1p mutation altered the intracellular amino acids contents, in which cysteine might be a biomarker in response to Pdr1p mutation under 2-PE stress. The findings help to elucidate the molecular mechanisms for 2-PE stress tolerance by Pdr1p mutation in *S. cerevisiae*, identify key metabolic pathway responsible for 2-PE stress tolerance.

Keywords *Saccharomyces cerevisiae*, 2-Phenylethanol stress, Pdr1p mutation

Background

2-Phenylethanol (2-PE) is an aromatic alcohol with a rose-like fragrance that is widely used in various fields such as food, medicine, and fragrance industries [1]. With increasing concern about climate change, it is of great significance to develop green, sustainable, and efficient technologies to produce 2-PE [2]. At present, *Saccharomyces cerevisiae* is one of the most promising microorganisms for the biosynthesis of 2-PE [3]. However, 2-PE has a strong inhibitory effect on *S. cerevisiae* as its structure [4]. It results in the maximum concentration of 2-PE below 6.3 g/L, which is far below the level required for industrial fermentation.

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Pdr1p is a central multidrug-resistant transcription factor in *Candida glabrata* [5]. *C. glabrata* acquires azole resistance through gain-of-function (GOF) mutations in Pdr1p [6, 7]. GOF mutation usually is single amino acid substitution mutations in the gene encoding Pdr1p. These mutations yield a GOF phenotype and lead to the elevated transcription of downstream target genes [8]. GOF alleles of Pdr1p have been extensively studied in azole resistance of *C. glabrata* [9]. In addition, the mutant allele, Pdr1p (R821H), conferred resistance to high iron, copper and manganese medium in *S. cerevisiae* [10]. And Pdr1p^{F815S} also showed increased resistance to organic solvent in *S. cerevisiae* [11]. Currently, it has been demonstrated that Pdr1p mutation (C862R) can substantially increase the 2-PE stress tolerance of *S. cerevisiae* [12]. But little is known of how Pdr1p is normally regulated [13].

Therefore, the present study investigated the growth, physiological, and biochemical parameters of Pdr1p mutation in *S. cerevisiae* under 2-PE stress. Furthermore, transcriptomic analysis was used to reveal the global gene response in response to Pdr1p mutation at 2-PE stress. The findings shed light on the molecular mechanisms for Pdr1p mutation in the 2-PE stress response, which would provide the target for constructing stress-resistant yeast cell factories.

Results

Changes in growth and metabolism conferred by Pdr1p mutation at 2-PE stress

To investigate the effect of the Pdr1p mutation on the growth and fermentation of *S. cerevisiae* under 2-PE stress, in this study, *PDR1*_862 strain and 5D strain were cultured under 3.5 g/L and 0 g/L 2-PE, respectively. As shown in Fig. 1A, no differences were observed in the growth of *PDR1*_862 and 5D strains at 0 g/L 2-PE. Under 3.5 g/L 2-PE stress, the OD_{600} of *PDR1*_862 and 5D strains was 21.25 and 13.8, respectively, which were 27% and 50% lower than the OD_{600} in the absence of 2-PE stress, respectively (Fig. 1B). Notably, 3.5 g/L 2-PE substantially inhibited the growth of the yeast strains. However, the maximum OD_{600} of strain *PDR1*_862 under 3.5 g/L 2-PE stress was 54% higher than that of 5D, and its growth rate was 11% higher than that of 5D (Table 1). Similarly, the maximum biomass dry weight of strain *PDR1*_862 under 3.5 g/L 2-PE stress (7.16 mg/mL) increased by 45.05% than that of the 5D strain. These results indicated that the Pdr1p mutation markedly

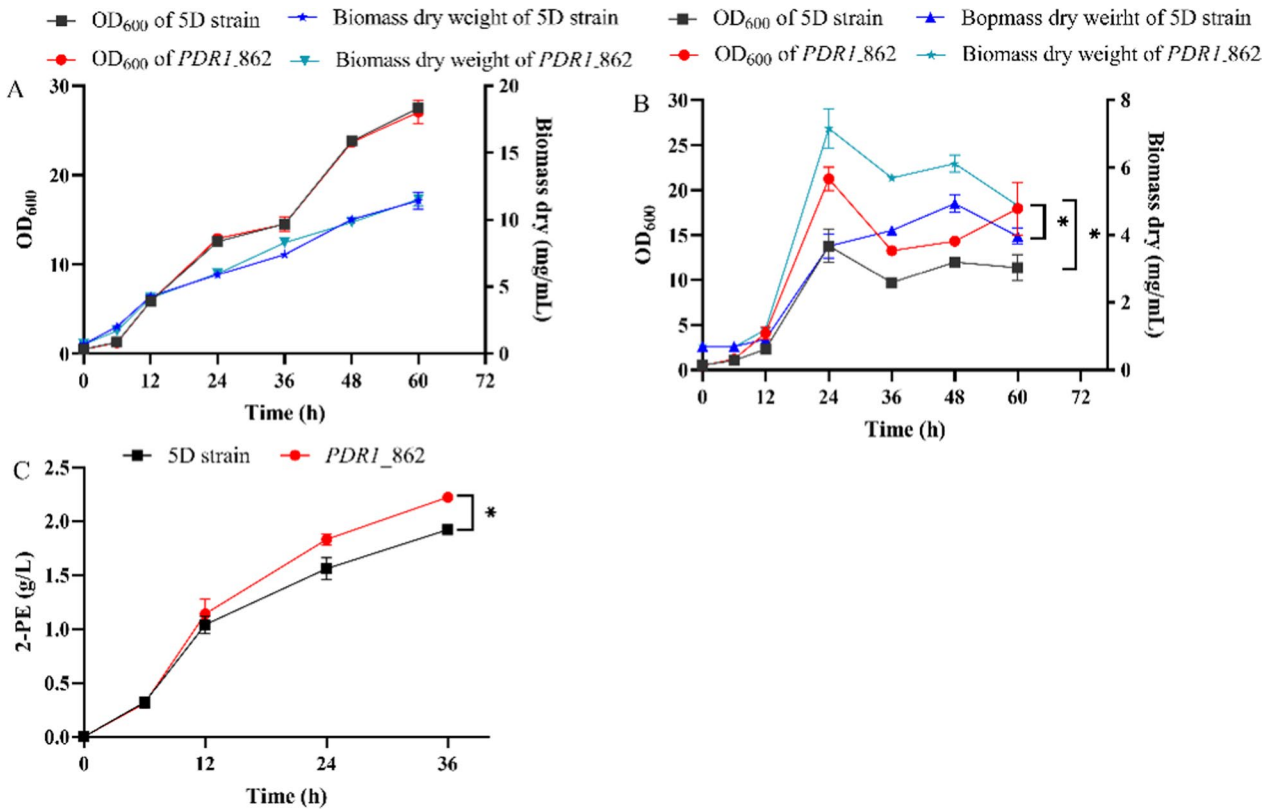

Fig. 1 The OD_{600} and biomass dry weight of *PDR1*_862 and 5D strains at 0 g/L (**A**) and 3.5 g/L 2-PE (**B**), and 2-PE concentration (**C**) of *PDR1*_862 and 5D strains. * Represents $p < 0.05$, as determined by Student's *t*-test

Table 1 Effect of the mutant of Pdr1p on the fermentation parameters

Strains	Growth rate (h^{-1})	Glucose consumption rate (g/L h)	Maximum ethanol concentration (g/L)
5D	0.087 ± 0.006	0.75 ± 0.006	10.93 ± 0.99
*PDR1*_862	0.097 ± 0.003	0.76 ± 0.004	8.60 ± 0.28

enhances the growth of *S. cerevisiae* under 2-PE stress. However, no difference was observed in the glucose consumption between *PDR1*_862 and 5D strains under 2-PE stress. Despite this, some differences were noted in their ability to synthesize ethanol: under 3.5 g/L 2-PE stress, the maximum ethanol concentration and ethanol synthesis rate of the *PDR1*_862 mutant strain were 21% and 22% lower than those of the 5D strain, respectively. Therefore, the growth of the *PDR1*_862 strain increased while the rate of ethanol synthesis decreased under 2-PE stress.

Effect of Pdr1p mutation on 2-PE content of *S. cerevisiae*

Pdr1p mutation enhanced the adaptability of *S. cerevisiae* to 2-PE stress, which led us to question if this enhancement would help to improve the ability of *S. cerevisiae* to produce 2-PE from L-Phe through Ehrlich pathway. Therefore, this paper compared the differences in the ability of *PDR1*_862 mutant and 5D strains to synthesize 2-PE. As shown in Fig. 1C, there was no difference in 2-PE concentration between *PDR1*_862 and 5D during early fermentation (0–6 h). However, at 12 h, the 2-PE concentration of *PDR1*_862 increased by 10% than that of 5D strain when fermented for 24 h, the 2-PE concentration of *PDR1*_862 significantly increased by 17% than that of 5D strain. After 36 h, the 2-PE concentration produced by strain *PDR1*_862 increased to 2.22 g/L, which was 16% higher than that of the 5D strain (1.92 g/L). These results demonstrated that the increased resistance to 2-PE stress in strain *PDR1*_862 was conducive to the improvement of 2-PE concentration.

In summary, the Pdr1p mutation reduced ethanol synthesis rate of yeast cells under 2-PE stress but enhanced the growth and the ability of the yeast cells to synthesize 2-PE.

Global gene response at the transcriptional level in response to Pdr1p mutation at 2-PE stress

To further investigate the underlying molecular mechanisms of Pdr1p mutation in the 2-PE stress response, RNA-Seq was conducted to compare global gene expression in *S. cerevisiae PDR1*_862 and 5D strains at 0 g/L and 3.5 g/L 2-PE. As shown in Fig. 2A, the samples exhibited high intragroup repeatability and large intergroup differences. A total 1524 genes were differentially expressed in the four groups, and 1420 DEGs were identified in the 5D vs 5DPE (749 upregulated and 671 downregulated); 711 DEGs were identified in the PDR vs PDRPE (409 upregulated and 302 downregulated); 120 DEGs were found in the 5DPE vs PDRPE (35 upregulated and 85 downregulated); 7 genes were upregulated between the 5D and PDR group (Fig. 2B). Among the DEGs, 4 genes were identified in all four groups, and eight DEGs were present only in 5DPE vs PDRPE (Fig. 2C), with three genes (*SNZ1*, *GCV1*, and *TNA1*) significantly upregulated and five genes (*SNQ2*, *CPA1*, *ROX1*, *MET32*, and a gene with unknown function) significantly downregulated. These genes might be the targets for that Pdr1p mutation conferred to 2-PE stress. Among the eight genes, some genes, such as *GCV1*, *MET32*, *CPA1*, have been previously shown to be associated with the amino acid metabolism [14–16].

To further analyze pathways enriched by the DEGs, the total DEGs were subjected to KEGG pathway enrichment analysis. KEGG analysis revealed that the DEGs in the 5D vs 5DPE group were enriched in ribosomes, cyanoamino acid metabolism, purine metabolism, thiamine metabolism, fructose and mannose metabolism, nitrogen metabolism, amino sugar and nucleotide sugar metabolism, aminoacyl-tRNA biosynthesis, propionate metabolism, pyruvate metabolism, glycolysis/gluconeogenesis, unsaturated fatty acid biosynthesis, fatty acid elongation. However, Pdr1p mutation significantly altered the metabolic pathways in *S. cerevisiae* under 2-PE stress. The significant metabolic pathways enriched in 5DPE vs PDRPE were sulfur metabolism, folate one-carbon pool, yeast meiosis, glyoxylate and dicarboxylic acid metabolism, ABC transporter protein, glycine/serine/threonine metabolism (Fig. 3B). Among the significant enrichment pathways, sulfur metabolism, glyoxylate and dicarboxylic acid metabolism, and glycine/serine/threonine metabolism were closely related to amino acid metabolism (Fig. 3C). Therefore, the amino acid metabolism might subject to perturbation by Pdr1p mutation, and the intracellular amino acid concentrations would be changed. Of these metabolic pathways, sulfur metabolism displayed the most significant enrichment, which was inextricably bound to cysteine metabolism and glycine, serine, and threonine metabolism. Amino acid metabolism involved in alleviating stress [17, 18], such as acid stress, oxidative stress.

Suppression of sulfur metabolism conferred by Pdr1p mutation

Compared to those in the absence of 2-PE, the transcription levels of the ATP sulfurylase (*MET3*, log2FC = +2.48), APS kinase (*MET14*, log2FC = +3.40), PAPS reductase (*MET16*, log2FC = +2.94), and sulfite

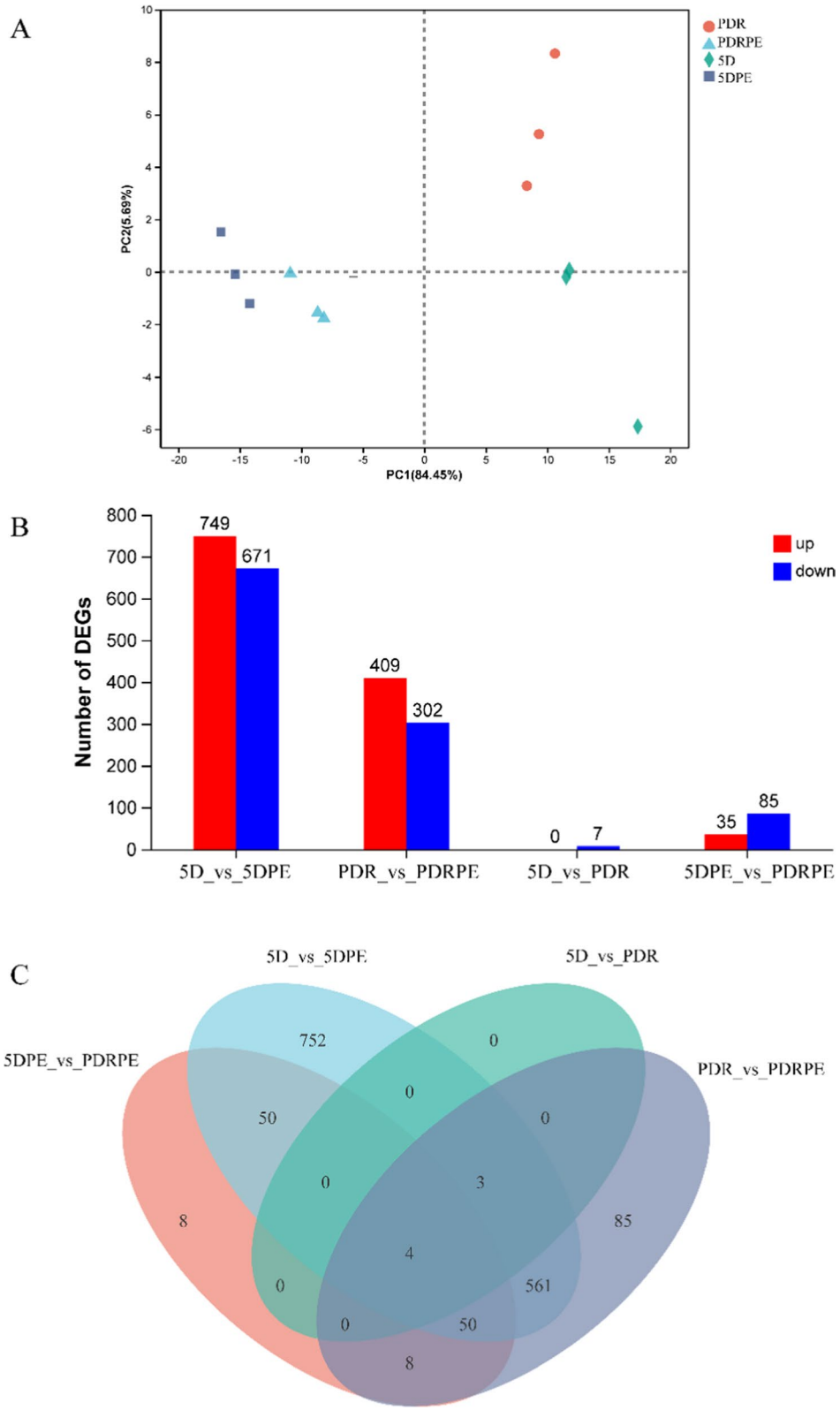

Fig. 2 Global analysis of transcriptomes and DEGs. **A** PCA analysis. **B** Differential statistics between groups, red represents upregulation, green represents downregulation. **C** Venn analysis of differential genes in different groups

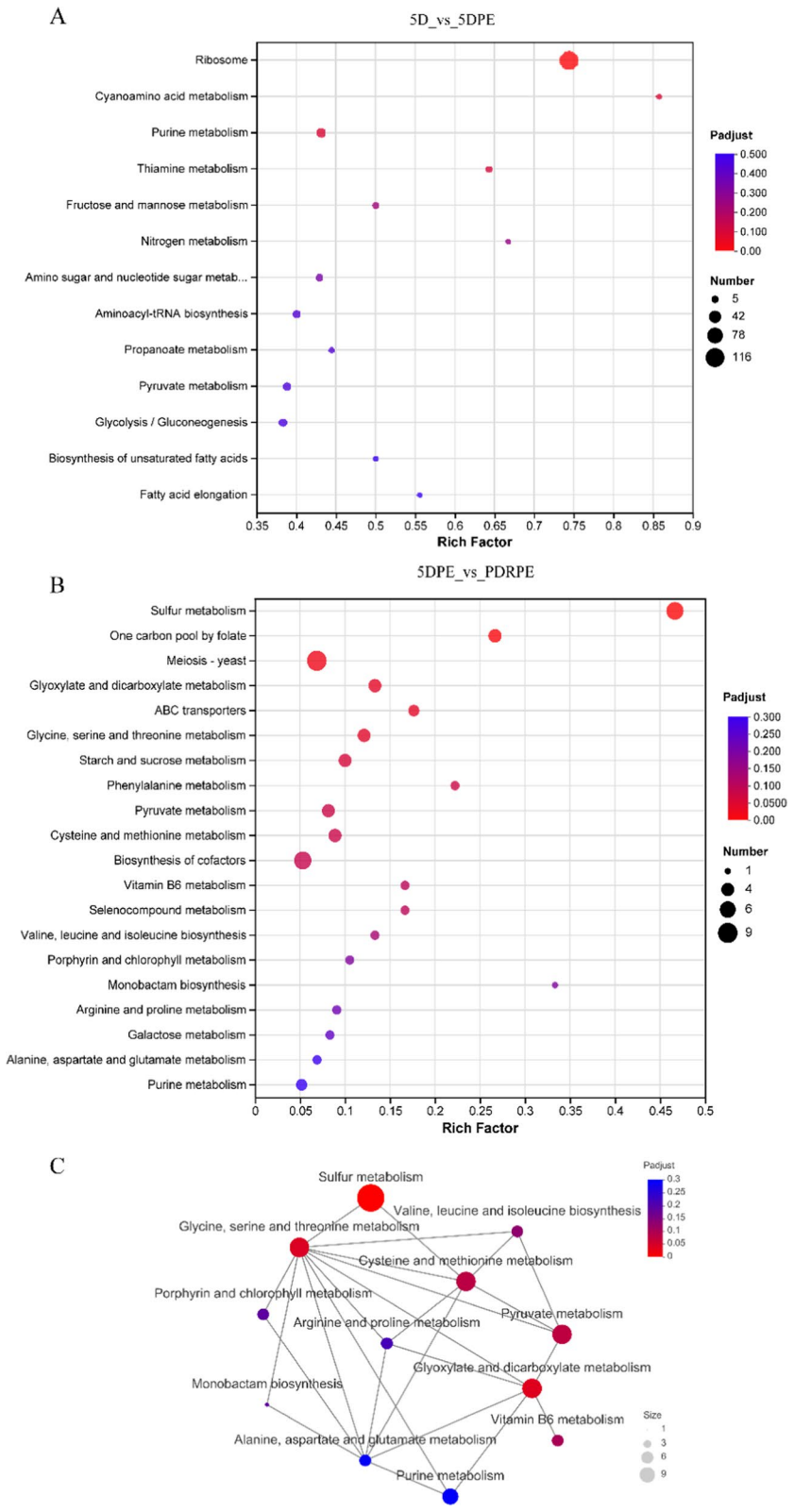

Fig. 3 The KEGG enrichment analysis of the DEGs in 5DPE_vs_PDRPE (**A**) and 5DPE_vs_PDRPE (**B**), and the network of the enrichment pathways (**C**)

reductase (*MET10*, log2FC = +1.67) genes involved in sulfur metabolism in 5D strain were significantly upregulated (Fig. 4); for strain *PDR1*_862, there was no difference of the above genes between 0 and 3.5 g/L 2-PE. However, compared to those of 5D strain at 3.5 g/L 2-PE, the transcription levels of the ATP sulfurylase (*MET3*, log2FC = −2.04), APS kinase (*MET14*, log2FC = −2.41), PAPS reductase (*MET16*, log2FC = −1.25), and sulfite reductase (*MET10*, log2FC = −1.33) genes were significantly downregulated in *PDR1*_862 strain. In conclusion, 2-PE stress enhanced transcription of the genes related to sulfur metabolism pathway in *S. cerevisiae*, and this transcriptional enhancement was attenuated by the Pdr1p mutation.

In addition, hydrogen sulfide (produced from sulfur metabolism) and *O*-acetylhomoserine were converted into cysteine by enzymes such as *O*-acetylserine sulfhydrylase (*MET17*) [19, 20]. 2-PE stress significantly upregulated the transcription of *MET17* (log2FC = +1.67) in strain 5D. However, compared with that of 5D strain, the transcription level of *MET17* was significantly downregulated in the *PDR1*_862 strain under 2-PE stress. Therefore, under 2-PE stress, Pdr1p mutation might reduce the synthesis of L-cysteine, which is an important sulfur-containing amino acid in yeast cells.

Enhancement of one-carbon pool by folate conferred by Pdr1p mutation

Folate concentrations of yeast cells are limiting, which can mediate one-carbon metabolism such as serine glycine interconversion and de novo purine synthesis. Compared to those in the absence of 2-PE, the transcription levels of formyltransferase (*ADE17*, log2FC = −162), glycine decarboxylase (*GCV1*, log2FC = −0.16), glycine hydroxymethyltransferase (*SHM2*, log2FC = −1.64), and methenyltetrahydrofolate cyclohydrolase (*MIS1*, log2FC = −0.21), which are all involved in the folate one-carbon pool, were downregulated in 5D strain under 2-PE stress. However, the transcription levels of formyltransferase (*ADE17*, log2FC = +1.03), glycine decarboxylase (*GCV1*, log2FC = +1.24), and glycine hydroxymethyltransferase (*SHM2*, log2FC = +1.30) were significantly upregulated in the *PDR1*_862 strain compared with those in the 5D strain under 2-PE stress. 2-PE inhibited the expression of genes in the folate one-carbon pool pathway in *S. cerevisiae*, the Pdr1p mutation alleviated the transcriptional suppression of these genes. Folate one-carbon pool pathway was associated with the interconversion between glycine and serine, which also related to other amino acid metabolism (Fig. 4).

Enhancement of the Ehrlich pathway conferred by Pdr1p mutation

Notably, 2-PE stress significantly reduced the transcription of aromatic amino acid aminotransferase (*ARO9*, log2FC = −2.05) and aromatic amino acid decarboxylase (*ARO10*, log2FC = −2.21) of the Ehrlich pathway in *S. cerevisiae*, whereas no significant change was found in the transcription of the above two genes in the *PDR1*_862 strain (Fig. 5). However, the transcription levels of *ARO9*

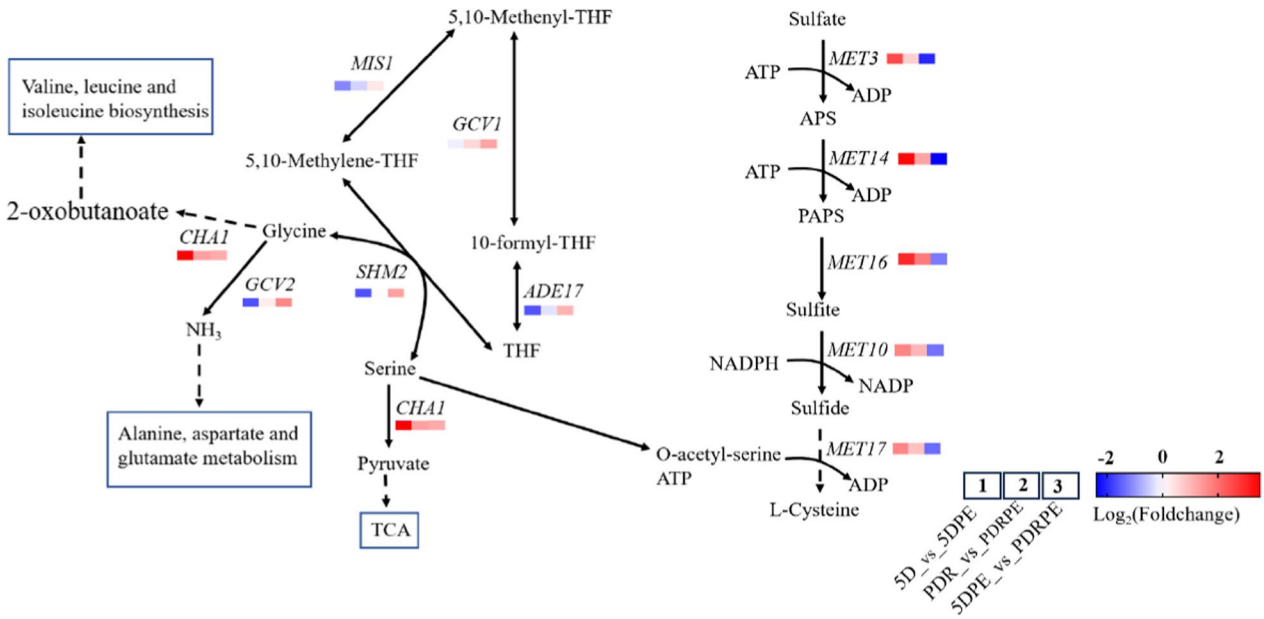

Fig. 4 Changes of differentially expressed genes in sulfur metabolism and one-carbon pool by folate

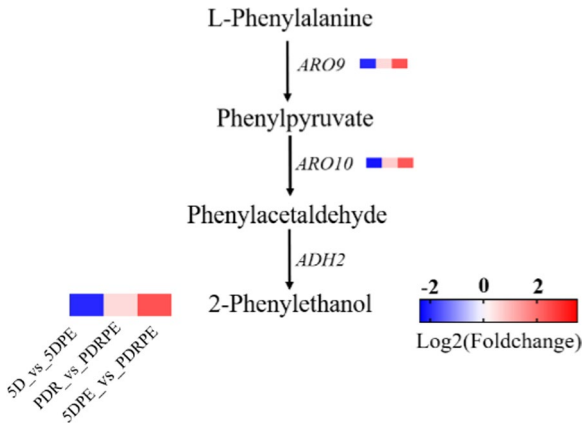

Fig. 5 Changes of differentially expressed genes in Ehrlich pathway

and *ARO10* were significantly upregulated 2.39- and 2.30-fold, respectively, in 5DPE vs PDRPE. Thus, 2-PE stress repressed the transcription of the genes encoded in the Ehrlich pathway in *S. cerevisiae*, but the Pdr1p mutation increased the transcription level of the genes in this pathway. Pdr1p mutation enhance 2-PE synthesis through the Ehrlich pathway in *S. cerevisiae*.

Validation of selected genes by qPCR

To validate RNA-seq data, 12 genes were selected to analyze the expression level by qPCR. As shown in Fig. 6, 2-PE stress increased the expression levels of *MET3*, *MET10*, *MET14*, *MET16*, and *MET17* in *S. cerevisiae*, and the transcription levels of these genes was still upregulated by 2-PE stress after Pdr1p mutation. However, their expression was significantly downregulated compared with that of the 5D strain. 2-PE stress significantly downregulated the expressions of the *ADE17*, *SHM2*, *GCV1*, and *GCV2* genes. Although 2-PE stress still downregulated the transcription of these genes after Pdr1p mutation, they were significantly upregulated compared with those of the 5D strain. The expression pattern of the abovementioned genes was consistent with that of the RNA-seq data.

Effect of Pdr1p mutation on 2-PE content of intracellular free amino acids

The above results indicated that the amino acid metabolism was subjected to disturbance. Next, intracellular free amino acids contents of the *PDR1*_862 and 5D strains at 0 g/L and 3.5 g/L 2-PE were analyzed. Table 2 shows the results of intracellular free amino acids. The total intracellular amino acid content of the 5D strain at 3.5 g/L 2-PE decreased by 13% than that at 0 g/L 2-PE, whereas the contents of serine, glutamate, alanine, and cysteine increased by 14%, 59%, 20%, and 37% than those in the absence of 2-PE stress, respectively. Similarly, the total intracellular amino acid content of the *PDR1*_862 strain at 3.5 g/L 2-PE decreased by 14% than that at 0 g/L 2-PE; whereas, the levels of aspartic acid, serine, glutamate, alanine, lysine, and arginine were 1.3, 2.03, 3.00, 2.46, 2.84, and 2.26 times higher, respectively, than those in the absence of 2-PE stress. However, under 2-PE stress, compared with that of the 5D strain, the intracellular contents of all free amino acids in the *PDR1*_862 strain were reduced (except for that of lysine), and the total intracellular amino acid content of the *PDR1*_862 strain was 31% lower than that of the 5D strain. These results demonstrated that 2-PE stress impaired intracellular amino acids homeostasis in *S. cerevisiae*, and Pdr1p mutation further reduced the intracellular amino acids concentration.

Subsequently, to further analyze the differences in the free amino acid contents among the four groups, principal component analysis was performed. As shown in Fig. 7A, the first two principal components (PC1, PC2) accounting for 84% of all samples revealed most information of the 17 FAAs. Among them, PC1 and PC2 accounted for 47.4% and 36.4% of the total variance, respectively. The scatter points showed that the samples were classified into four different groups, indicating that Pdr1p mutation had a significant effect on the contents of 17 free amino acids.

In order to screen potential marker amino acids among the 17 free amino acids, and partial least squares discriminant analysis (PLS-DA) was used. As shown in Fig. 7B, the scores of PLS-DA were 83%, and the samples were well separated. The marker amino acids were identified according with variable importance in the projection (VIP), and VIP > 1.0 is usually used as the selection criterion. The VIP values of cysteine, serine, glutamate, and histidine were 1.31, 1.26, 1.23, and 1.06, respectively (Fig. 7C), suggesting that cysteine was one of the most important amino acids in response to 2-PE stress, followed by serine, glutamate, and histidine. L-Cysteine metabolism plays a vital role in the metabolism of other intracellular amino acids and is closely associated with glycine, serine, and threonine metabolism; pyruvate metabolism; arginine and proline metabolism; valine, leucine, and isoleucine biosynthesis; and alanine, aspartic acid, and glutamate metabolism. It has been demonstrated that cysteine plays a significant role in the resistance against oxidative stress [21].

2-PE molecular docking

Although the mechanism by which Pdr1p mutation enhanced 2-PE stress tolerance is well understood, the molecular mechanisms of how mutant Pdr1p responds to 2-PE remain unknown. It has been demonstrated that

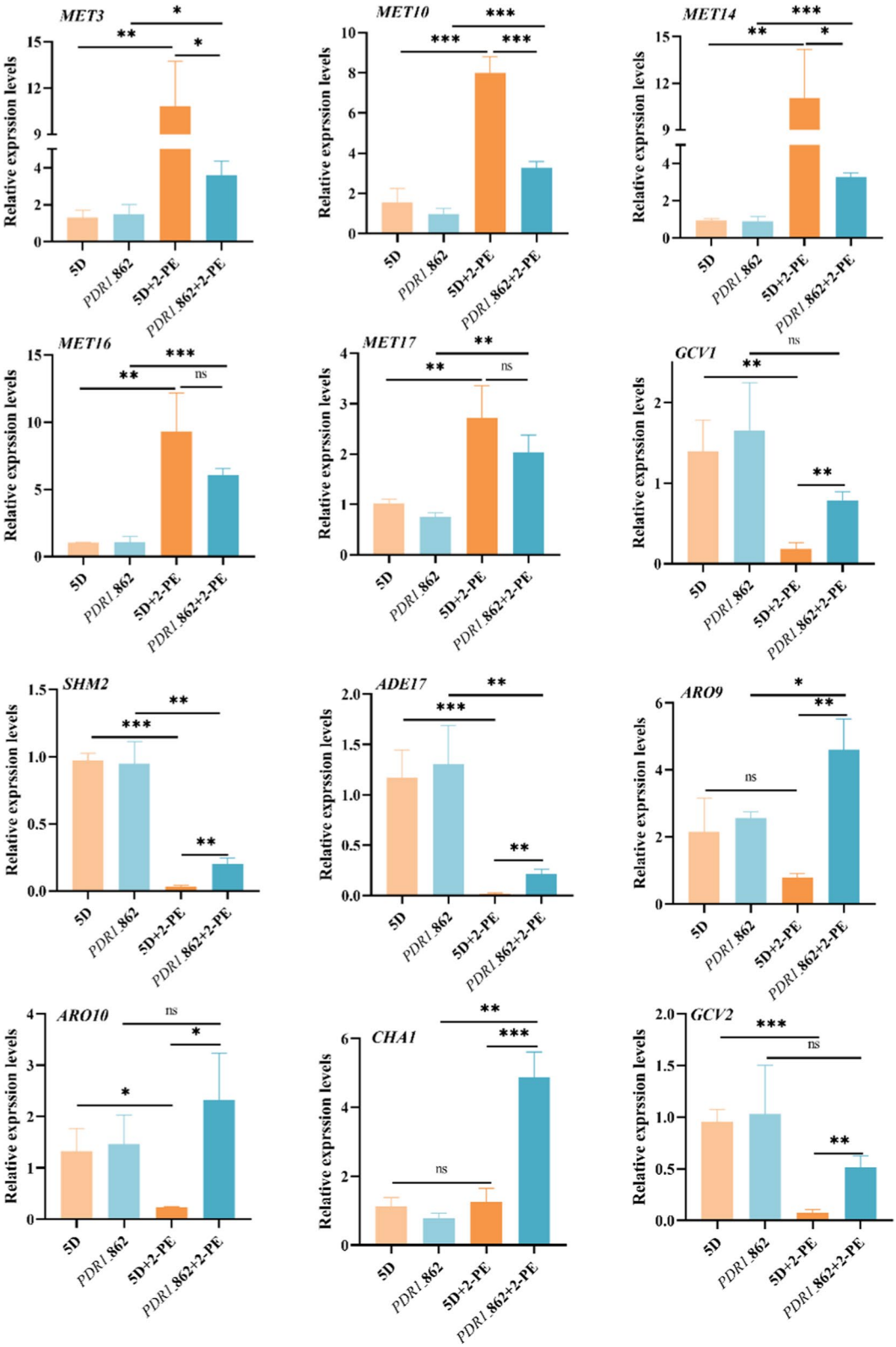

Fig. 6 Transcription levels of wild strain and *PDR1*_862 strain at 0 g/L 2-PE and 3.5 g/L 2-PE. * Represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$, ns represents no significance

Table 2 Free amino acids content of *PDR1*_862 and 5D strains at 0 g/L and 3.5 g/L 2-PE

Amino acids	A1: 5D (mg/g)	A4: 5DPE (mg/g)	A7: PDR (mg/g)	A10: PDRPE (mg/g)
Asp	5.104 ± 0.672	2.396 ± 0.126	1.713 ± 0.668	2.239 ± 0.143
Thr	6.890 ± 0.0.789	4.475 ± 0.357	2.514 ± 0.653	1.565 ± 0.234
Ser	5.743 ± 0.477	6.528 ± 0.328	2.125 ± 0.120	4.315 ± 0.300
Glu	17.076 ± 0.226	27.145 ± 0.110	7.826 ± 1.118	23.498 ± 0.076
Gly	6.243 ± 0.500	2.691 ± 0.077	2.325 ± 0.003	2.038 ± 0.017
Ala	4.613 ± 1.102	5.523 ± 0.053	2.823 ± 0.212	6.952 ± 0.146
Cys	7.570 ± 1.160	10.398 ± 0.091	10.190 ± 0.212	6.274 ± 0.024
Val	3.905 ± 0.633	3.171 ± 0.796	2.235 ± 0.025	2.193 ± 0.081
Met	9.208 ± 0.371	7.688 ± 0.278	12.176 ± 0.404	3.307 ± 0.594
Ile	21.113 ± 1.039	22.206 ± 1.379	27.918 ± 0.744	9.426 ± 0.160
Leu	8.051 ± 0.151	5.063 ± 0.430	6.183 ± 0.505	2.476 ± 0.025
Tyr	4.957 ± 0.955	4.005 ± 0.454	4.482 ± 0.136	1.479 ± 0.114
Phe	2.588 ± 0.739	2.715 ± 0.344	3.199 ± 0.233	0.708 ± 0.186
His	6.218 ± 0.173	2.610 ± 0.234	3.340 ± 0.477	2.879 ± 0.123
Lys	16.209 ± 0.453	8.509 ± 0.011	3.470 ± 0.179	9.849 ± 0.057
Arg	15.131 ± 0.370	11.687 ± 0.152	3.737 ± 0.050	8.436 ± 0.057
Pro	8.617 ± 1.350	3.001 ± 0.073	7.452 ± 0.694	2.000 ± 0.121
Total	149.232	129.808	103.704	89.628

Pdr1p directly binds to various xenobiotics and drugs [5]. We hypothesized that Pdr1p might also directly bind to 2-PE, and the Pdr1p mutation would change its molecular conformational relationship with 2-PE. In this study, molecular docking was performed to construct the complex model of Pdr1p (Fig. 8A). A certain number of hydrogen bonds were formed between the Pdr1p and the hydroxyl group of 2-PE. 2-PE bound to the internal cavity of Pdr1p with a low free energy, resulting in the formation of a relatively stable complex between 2-PE and Pdr1p. The binding energy between Pdr1p or Pdr1p ^{C862R} and 2-PE was −5.4 kcal/mol. Compared to Pdr1p, the interaction between the mutant of C862R and 2-PE remained unchanged (Fig. 8B). The results showed that Pdr1p and 2-PE bind to form a complex in response to 2-PE stress, but Pdr1p mutation has no effect on the molecular conformational relationship of the complex. Pdr1p might have multiple interfaces to control activity of the Pdr1p mediating stress resistance [22].

Discussion

In this sturdy, at 2-PE stress, the Pdr1p mutation increased the transcription level of the genes in the Ehrlich pathway, 2-PE content of *PDR1*_862 strain increased by 16% than that of 5D strain. However, the ethanol concentration of *PDR1*_862 strain decreased than that of the 5D strain under 2-PE stress. 2-PE was biosynthesized through the Ehrlich pathway, and NADH was needed for final reduction step in the pathway [23], while ethanol was synthesized at the expense of NADH. Therefore, under 2-PE stress, Pdr1p mutation reduced ethanol synthesis, which might increase the NADH availability to synthesize 2-PE. It has been demonstrated that the efficiency of 2-PE biosynthesis is often affected by an insufficient supply of NADH [24]. Wang et al. developed a NADH self-sufficient system for 2-PE synthesis, which resulted in 3.8-fold increase of 2-PE biocatalyst efficiency [25].

In addition, four molecules of NAD(P)H are consumed when one molecule of sulfate is reduced to produce hydrogen sulfide during sulfur metabolism [26]. 2-PE stress enhanced the transcription levels of the genes related to sulfur metabolism in 5D strain, which might increase the flux of sulfur metabolism, and in turn, increased the consumption of NAD(P)H. However, under 2-PE stress, the Pdr1p mutation attenuated their transcription and decreased the consumption of NAD(P)H. This suggested that Pdr1p mutation actually saves cellular NAD(P)H consumption through weakening sulfur metabolism, which might increase NAD(P)H availability for 2-PE biosynthesis.

The regulation of 2-PE stress tolerance by Pdr1p mutation in *S. cerevisiae* is global and complex. Pdr1p mutation could substantially affect multiple intracellular metabolic pathways, such as L-Phe metabolism, folate one-carbon pool metabolism, and sulfur metabolism. These metabolic pathways are closely related to intracellular amino acid metabolism [14, 27]. In this study, Pdr1p mutation altered the intracellular amino acids contents. Among the amino acids, L-cysteine was the

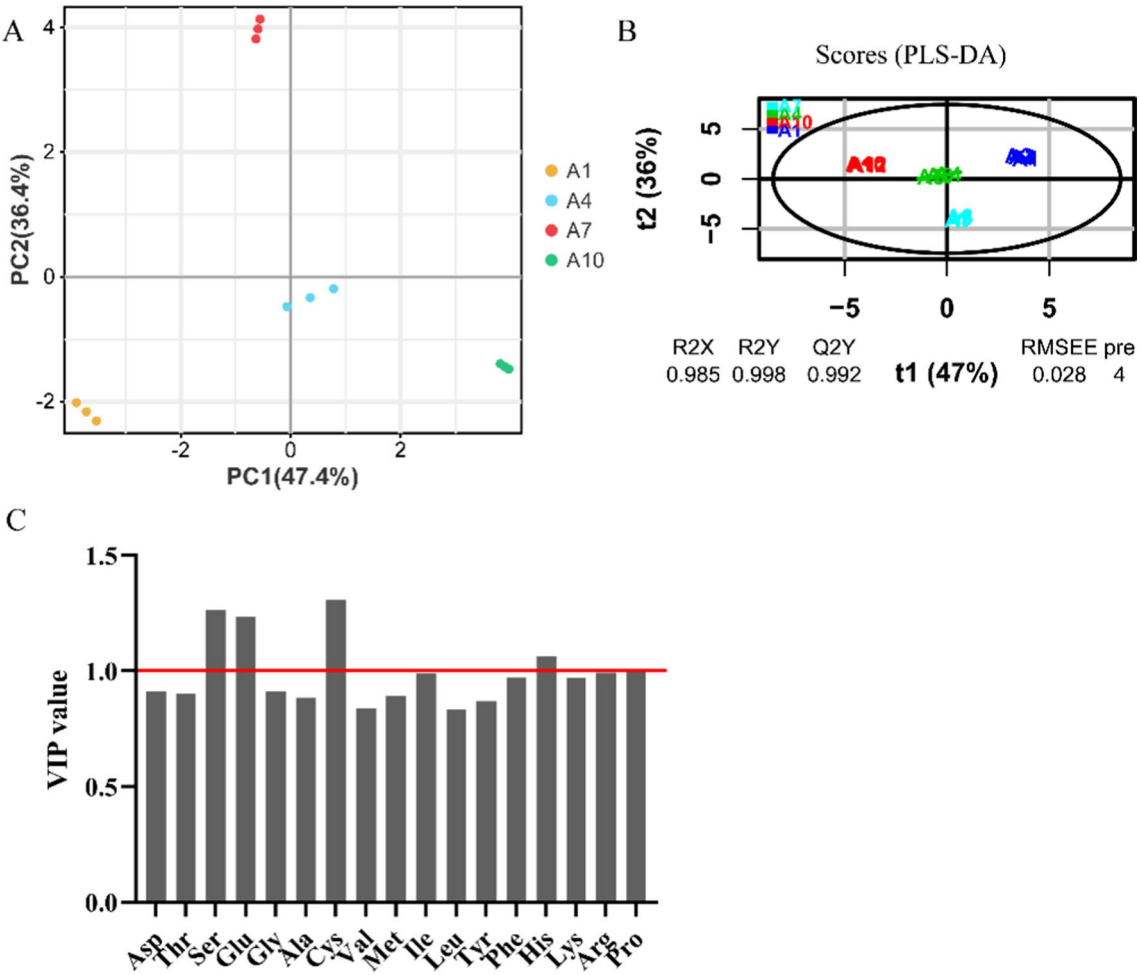

Fig. 7 Comparison of free amino acids between *PDR1*_862 and 5D strain at 0 g/L and 3.5 g/L 2-PE stress. **A** and **B** PCA score plot and the score plot by PLS-DA analysis of free amino acids. **C** The VIP values of the amino acids

key amino acid in response to Pdr1p mutation under 2-PE stress. Amino acid metabolism involved in alleviating stress [17, 18], such as acid stress, oxidative stress. It has been demonstrated that 2-PE stress induces intracellular ROS accumulation in *S. cerevisiae*, Pdr1p mutation significantly reduces intracellular ROS. L-Cysteine plays an important role in the defence against oxidative stress. Therefore, L-cysteine might be the biomarker of Pdr1p mutation in response to 2-PE stress. Exogenous amino acids might enhance the ability of *S. cerevisiae* to survive at 2-PE stress.

S. cerevisiae, as one of the most promising microorganisms for 2-PE biosynthesis, is a popular strain for the research on the 2-PE biosynthesis. 2-PE has a strong inhibitory effect on the yeast cells, which makes it difficult to realize scale-up production of 2-PE, while currently, the limited understanding of the 2-PE stress tolerance mechanism in yeast cells hinders the rational design and industrial application of yeast cells with high 2-PE productivity [28]. The Pdr1p mutation enhanced growth phenotype under 2-PE stress, simultaneously improved the production of 2-PE, which is important for achieving industrial production of 2-PE. This would have significant implications for the targeted design, integration of stress-resistant genetic circuits, and reprogramming of *S. cerevisiae* cell factories for the biosynthesis of 2-PE.

Conclusion

The Pdr1p mutation enhanced growth phenotype under 2-PE stress, simultaneously improved the production of 2-PE. Based on the results of transcriptome analysis, the Pdr1p mutation attenuated the sulfur metabolism, enhanced folate one-carbon pool and Ehrlich pathways. These pathways are associated with the amino acid metabolism. Pdr1p mutation was shown to be indeed affected the intracellular amino acids content. Pdr1p mutation reduced the intracellular free amino acid

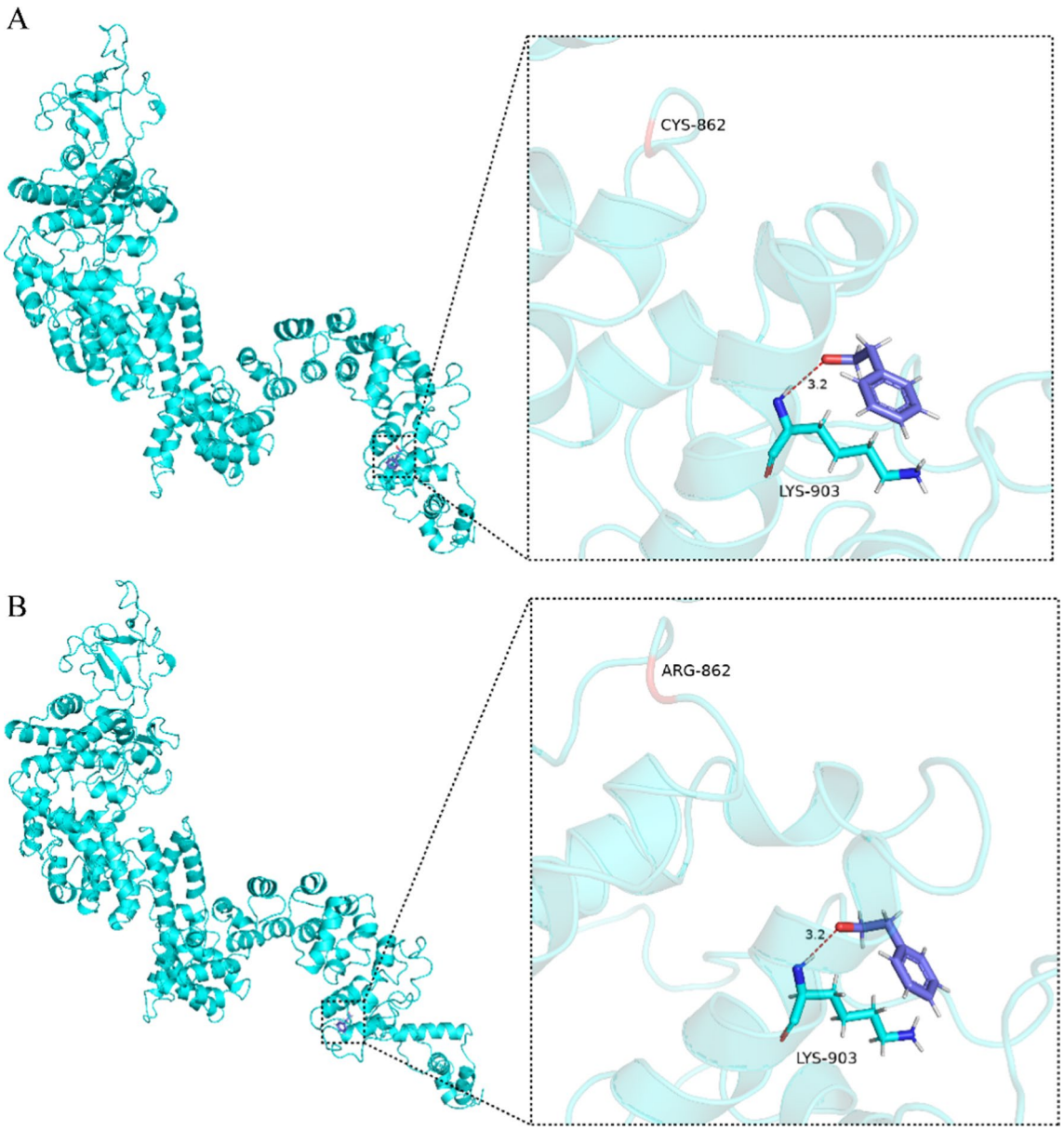

Fig. 8 Molecular docking results between 2-PE and Pdr1p (**A**)/Pdr1p ^{C862R} (**B**)

concentration under 2-PE stress, among the amino acids, cysteine was the key amino acids in response to Pdr1p mutation under 2-PE stress.

Materials and methods

Strains and medium

The *S. cerevisiae PDR1*_862 and *S. cerevisiae* CEN. PK113-5D (5D strain) were used in this study [12]. YEPD medium (20 g/L peptone, 10 g/L yeast extract, 20 g/L glucose) was used to cultivate *S. cerevisiae*, and 2-PE were added at concentration of 3.5 g/L or 0 g/L if needed. The 2-PE synthetic medium consisted of glucose (30 g/L), L-phenylalanine (4.5 g/L), $MgSO_4$–$7H_2O$ (0.5 g/L), and YNB (without $(NH_4)_2SO_4$) (1.7 g/L).

RNA extraction

The *PDR1*_862 mutant and 5D strain were cultured at 30 °C, 200 rpm for 12 h, the cultures were inoculated into YEPD containing 0 g/L or 3.5 g/L 2-PE with an initial OD_{600} of 0.5. Three biological replicates were set up. The cultures were collected, and total RNA was extracted. Then RNA quality was determined by 5300

Bioanalyser (Agilent) and quantified using the ND-2000 (NanoDrop Technologies). Only high-quality RNA sample ($OD_{260/280}$ = 1.8–2.2, $OD_{260/230} \geq 2.0$, RIN ≥ 6.5, 28S:18S ≥ 1.0, > 1 μg) was used to construct sequencing library. RNA purification, reverse transcription, library construction and sequencing were performed at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China).

Different expression analysis

To identify differential expression genes (DEGs) between two different samples, differential expression analysis was performed using the DESeq2 or DEGseq. DEGs with |log2FC|≧1 and FDR ≤ 0.05 (DESeq2) or FDR 0.001(DEGseq) were considered to be significantly different expressed genes. In addition, functional-enrichment analysis including GO and KEGG were performed to identify which DEGs were significantly enriched in GO terms and metabolic pathways at Bonferroni corrected *p*-value ≤ 0.05 compared with the whole-transcriptome background [29].

Detection of gene transcription levels

The total RNA of *PDR1*_862 mutant and 5D strain under 0 g/L or 3.5 g/L 2-PE were used to verify the gene transcription levels. The genomic DNA was removed from the total RNA via incubation at 42 °C for 2 min using the 4×gDNA wiper Mix. The RNA was then reverse-transcribed into cDNA using 5×HiScript II QRT SuperMix. This cDNA was used as a template for PCR amplification in a CFX96TM Real-Time PCR Detection System (Bio-Rad, USA) using ChamQ universal SYBR qPCR Master Mix, with *ENO1* as the internal reference [30].

Intracellular amino acids determination

The yeast cells were added to 8 mL of 40% ethanol/water solution + glass beads (cells:glass beads = 1:5) and then vortexed for 15 min. The mixture was centrifuged (8000×*g*, 5 min). A certain amount of sample was obtained via pipetting, and 4 mL of pure hydrochloric acid was added in a 1:1 volume ratio. After nitrogen-blowing and hydrolysis, the obtained sample was passed through a 0.22-μm filter. The contents of free amino acids were then detected using an amino acid analyzer (L8900) with a column size of 200 mm × 46 mm and a cationic exchange resin column for ion-exchange chromatography. The flow rate was 0.2 mL/min, the column was 55 °C, and the reaction chamber was 138 °C.

Structural prediction and analysis of Pdr1p

Discovery Studio 2019 was used to construct the Pdr1p model. Then CB-DOCK2 will conduct cavity detection based on artificial neural networks, and utilizes Autodock Vina for docking [31]. The analysis of the interaction forces between 2-PE and Pdr1p will be completed by the PLIP online server.

Analytical methods

The cell density (OD_{600}) was measured by spectrophotometer (752N, China). The concentration of 2-PE was measured by HPLC (Thermo Fisher Scientific, USA) according to Huili Xia et al. [12]. Ethanol content was detected by Biosensor Analyzer (Sieman Technology Co., Ltd, Shenzhen China).

Statistical and reproducibility

The data analysis and graphing were performed by GraphPad Prism 8 (GraphPad Software, USA). All experiments were conducted in three biological replicates.

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Author contributions

HL X, J D and X C designed the whole and wrote the final manuscript. DQ L and N S carried out all experiments, data collection, and manuscript editing. LI SG and R Z participated in data analysis.

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Availability of data and materials

All data generated or analyzed during this study are included in this manuscript..

Declarations

Competing interests

The authors declare no competing interests.

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