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OVEREXPRESSION OF GLUTAREDOXINS ENHANCES PHYSICOCHEMICAL STRESS TOLERANCE IN *SACCHAROMYCES CEREVISIAE*

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Abstract

Cellulosic biomass is a renewable substrate for bioethanol generation, but the industrial fermentation performance of *Saccharomyces cerevisiae* is severely reduced under physicochemical stresses. Eight glutaredoxin (GRX) genes from *S. cerevisiae* were individually overexpressed and evaluated for stress tolerance. Under heat stress at 42°C, strains overexpressing *Grx1*, *Grx2*, and *Grx5* exhibited 34.5%±0.2%, 19.6%±5.0%, and 28.0%±2.5% higher OD_{600} values at 72 h compared to the control strain. Under 1 g/L acetic acid, strains overexpressing *Grx1*, *Grx2*, *Grx6*, and *Grx7* showed 13.0%±4.0%, 8.3%±1.4%, 4.3%±0.5%, and 5.9%±1.5% increases in OD_{600}, respectively. ROS quantification revealed that *Grx1*-, *Grx2*-, and *Grx5*-overexpressing strains showed substantially lower intracellular ROS accumulation, with intracellular ROS levels of 36.2%±1.0%, 41.2%±1.7%, and 58.8%±0.8% relative to the control strain (empty POT1 plasmid) at 42°C. Transcriptional analysis further showed distinct stress-responsive expression patterns, with *Grx1* and *Grx2* exhibiting high basal transcription at 30°C, downregulation at 40°C, and re-induction under acetic acid stress.

Collectively, these findings demonstrate that overexpression of the glutaredoxin system improves redox homeostasis and significantly enhances yeast performance under physicochemical stress.

Key words: *Saccharomyces cerevisiae*; Glutaredoxin; Physicochemical stress; Redox homeostasis; Lignocellulosic biomass fermentation

Introduction

Green biomanufacturing (GB) through microbial fermentation uses industrial biotechnology to produce biofuels and biochemicals (Bian *et al*., 2021). GB offers a viable alternative to conventional petrochemical-based production, which is often associated with high fossil fuel consumption, greenhouse gas (GHG) emissions, toxic byproduct generation, heavy metal contamination, high energy input requirements, and wastewater pollution (Xu *et al*., 2018a; Xu *et al*., 2020a). From an industrial biotechnology perspective, improving host robustness is a prerequisite for translating laboratory-scale fermentation systems into economically viable biorefinery operations. In addition, large-scale biorefineries demand microbial platforms capable of maintaining metabolic stability under fluctuating environmental parameters. Therefore, improving intrinsic cellular robustness is not only a biological objective but also a process-engineering requirement for next-generation sustainable manufacturing systems. However, the pretreatment of lignocellulosic biomass, such as acid hydrolysis and alkaline pretreatment, frequently generates inhibitory compounds, including weak organic acids (Qin *et al.*, 2020). Among these inhibitors, acetic acid is particularly detrimental, as it diffuses into yeast cells (Zheng *et al.*, 2011). Several studies have demonstrated that acetate accumulation significantly suppresses yeast growth, reduces biomass formation, and inhibits ethanol fermentation efficiency (Xiao *et al*., 2025; Pampulha & Loureiro-Dias, 2000). Beyond growth inhibition, weak-acid stress also disrupts intracellular ATP homeostasis, alters membrane permeability, and increases maintenance energy demand, thereby lowering overall carbon conversion efficiency. At the cellular level, undissociated acetic acid penetrates the plasma membrane and dissociates in the cytosol, leading to intracellular acidification and proton accumulation. This triggers ATP-dependent proton extrusion, increases maintenance energy burden, and diverts metabolic flux away from biomass and ethanol production.

In SSF, cellulose serves as the main substrate, but the main problem is the incompatibility between the optimal temperature for cellulase activity (~55°C) and yeast growth (~30°C) (Farinas *et al.*, 2010). Fermentation temperature is influenced not only by external environmental factors but also by heat released during yeast metabolism (Xu *et al*., 2020b). An increase in fermentation temperature leads to the accumulation of ROS, which, in turn, causes oxidative stress (Xu *et al*., 2018b).

Engineering a thermotolerant yeast can significantly reduce operational costs and water required for cooling for the production of ethanol from cellulosic biomass. Operating fermentation at elevated temperatures (40–45°C) decreases the need for active cooling systems, which can account for approximately 15–30% of total utility costs in large-scale biorefineries. Studies and techno-economic analyses suggest that thermotolerance

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engineering may reduce overall production costs by 5–12%, primarily by lowering cooling energy consumption, reducing contamination risk, shortening fermentation times, and improving compatibility with simultaneous saccharification and fermentation (SSF) processes. Evolutionary engineering, genome shuffling, and modulation of global transcriptional networks are commonly employed for trait engineering in microbial hosts (Xu *et al.*, 2022). *Saccharomyces cerevisiae* typically shows poor growth under stress conditions. The thermo-acid-tolerant TAT12 strain carrying mutations in RAS2 and HSF1 has been developed through adaptive laboratory evolution and genome analysis (Salas-Navarrete *et al.*, 2022). However, thermotolerance engineering often compromises metabolic flux distribution, necessitating balanced redox optimization strategies to prevent unintended trade-offs.

GSH-dependent antioxidant system forms the core of cellular defense against hydroperoxides, electrophiles, and redox imbalance (Buchmann *et al*., 2020). GRXs are classified as dithiol (2-Cys) or monothiol (1-Cys) (Molina *et al.*, 2004). Eukaryotic redox homeostasis depends on the glutathione–glutaredoxin (GRXs) system. GRXs mediate deglutathionylation; consequently, they prevent ROS-induced protein damage under stressful conditions (Go & Jones, 2008). In our previous study, we demonstrated the role of glutaredoxins (GRXs) in coping with high temperatures by overexpressing *GRX5* (Cheng *et al.*, 2006; Xu *et al*., 2023). System-wide amplification of the glutaredoxin network may provide a coordinated redox buffering effect rather than single-gene protection, potentially enhancing stress adaptation under multifactorial industrial conditions.

Synthetic biology has enabled the design of modular redox circuits that can be transplanted into host cells to enhance stress resilience. In this study, all GRX genes—including *GRX1* (Glutaredoxin 1), *GRX2* (Glutaredoxin 2), *GRX5* (Glutaredoxin 5), *GRX6* (Glutaredoxin 6), *GRX7* (Glutaredoxin 7), and *GRX8* (Glutaredoxin 8)—from *S. cerevisiae* were overexpressed to evaluate their capacity to improve tolerance to physicochemical stress.

Materials and Methods

Strains, plasmids, and media: *Saccharomyces cerevisiae* BY4741 (his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) was used as the host strain. The POT1 plasmid contains the URA3 auxotrophic marker, which allows selection on SD-ura medium. The control strain (empty POT1 plasmid) refers to BY4741 transformed with the empty POT1 plasmid. The control strain and GRXs module expressing *S. cerevisiae* BY4741 strain was grown in SD-ura medium (synthetic defined medium lacking uracil). The bacterial strain was grown at 37°C in LB medium, and 100 μg/mL ampicillin or kanamycin was added as required by the experiment. Plasmids HCKan-P, HCKan-O, and HCKan-T were used for Golden Gate assembly gifted by Prof. Dai (Shenzhen Institutes of Advanced Technology, Shenzhen, China). Plasmids constructed in this study are listed in Table S1.

Table S1. Plasmids used in this study.

Plasmid	Host Strains	Reference
HCKan-P	*E. coil*	Guo *et al*., 2015
HCKan-O	*E. coil*	Guo *et al*., 2015
HCKan-T	*E. coil*	Guo *et al*., 2015
POT-1	*E. coil* and *S. cerevisiae*	Guo *et al*., 2015

DNA manipulation: The *E. coli* plasmid and yeast genomic DNA were extracted using the TIANprep Mini Plasmid Kit and TIANamp Yeast DNA Kit, respectively, purchased from TIANGEN (China). The cloning reagents, including restriction enzymes and Golden Gate assembly master mix, were purchased from New England Biolabs (Ipswich, MA, USA). 8 Grx coding sequences were obtained from SGD and amplified from gDNA. The PCR products were purified using the TIAN Quick Midi Purification Kit (TIANGEN). The genetic circuits of GRX genes were constructed with Golden Gate assembly master mix on the POT1 plasmid (Boonzaier *et al.*, 2025). The primer sequences are listed in the Supplementary Table S2. Each amplified Grx fragment was inserted into the POT1 vector (Fig. S2). The recombinant plasmids were transformed into BY4741. The strain transformed with the empty POT1 vector was used as the control.

Table S2. Primers used in this study.

Gene	Primer-F	Primer-R
GRX1	AGCGTGGGTCTCAGATG GTATCTCAAGAAACTATCAA	GTGCTGGGTCTCGGCTA ATTTGCAAGAATAGGTTCTA
GRX2	AGCGTGGGTCTCAGATG GAGACCAATTTTTCCTTCGA	GTGCTGGGTCTCGGCTA TGAAATACCGGCTTCAATAT
GRX3	AGCGTGGGTCTCAGATG TGTTCTTTTCAGGTTCCATC	GTGCTGGGTCTCGGCTA AGATTGGAGAGCATGCTGCA
GRX4	AGCGTGGGTCTCAGATG ACTGTGGTTGAAATAAAAAG	GTGCTGGGTCTCGGCTA CTGTAGAGCATGTTGGAAAT
GRX5	AGCGTGGGTCTCAGATG TTTCTCCCAAAATTCAATCC	GTGCTGGGTCTCGGCTA ACGATCTTTGGTTTCTTCTT
GRX6	AGCGTGGGTCTCAGATG ATACCTTCCAATAAGAGAAA	GTGCTGGGTCTCGGCTA ATTATTGGAAGGTTTTTCAC
GRX7	AGCGTGGGTCTCAGATG GCTATTGTTATAAACAAAAG	GTGCTGGGTCTCGGCTA GGCACTCTCAGATTGCGAAT
GRX8	AGCGTGGGTCTCAGATG TCTGCCTTTGTTACTAAAGC	GTGCTGGGTCTCGGCTA AGGCAGAAGCCCGATTTTAG

Note: Primers are designed for Golden Gate cloning. The upper sequence includes *Bsa* I recognition sites and assembly overhangs; the lower sequence is the gene-specific biding region for target *GRX* genes

RT-qPCR analysis: For RT-qPCR analysis, TRIzol reagent (TransGen Biotech, China) was used to isolate total RNA from yeast strains in this study. TransScript First-Strand cDNA Synthesis Kit (TransGen Biotech, China) and Top/Tip Green qPCR SuperMix were used for cDNA synthesis and reaction mixture preparation, respectively. The qPCR reaction was performed on a Roche LightCycler 96 (CA, USA). The gene of ACT1 was used to calculate the relative expression by applying the $2^{\wedge-\Delta\Delta Ct}$ formula (Vinje *et al*., 2023).

RT-qPCR transcriptional analysis: Total RNA was extracted from yeast cells under three different experimental conditions: (A) 30℃ (control), (B) 40℃ (heat stress), and (C) 30℃ with 1 g/L acetic acid. Yeast cultures were inoculated to an initial OD_{600} of 0.1 in YPD medium and incubated at 250 rpm for 12 h. Cells were harvested by centrifugation at 12,000 rpm for 5 min at 4℃, washed twice with sterile water, and used for RNA extraction and subsequent RT-qPCR analysis. Transcriptome sequencing was performed by BGI for preliminary exploration only, and these data will be reported in a separate study.

Shake-flask cultivation: Yeast cells were grown in uracil-deficient SD-medium containing 20 g/L dextrose, and heat stress was applied by growing at 42°C, while 1 g/L acetic acid was added to inflict acid stress. Pre-cultures were incubated overnight at 30°C and 200 rpm in 2 mL medium. Then, strains were grown in 250 mL flasks containing 30 mL fresh medium，sealed with a foam plug, and incubated at 200 rpm. The starter culture OD_{600} was 0.1. The strain grown for 72 h and OD_{600} was measured every 12 h. Samples were diluted to maintain readings within the linear range (0.2–0.8).

Qualitative spot assay for stress survival: For qualitative assessment of stress tolerance, cells of the control strain (empty POT1 plasmid), harboring empty POT1 plasmid and *GRX*-overexpressing strains (*GRX1*, *GRX2*, *GRX5*, *GRX6*, *GRX7*) were harvested at 24 h of cultivation. Cells were serially diluted in sterile ddH_2O, and 5 μL of each dilution was spotted onto SD-ura agar plates. For heat stress assays (Fig. 3a), serial dilutions from 10^{-1} to 10^{-4} were spotted, and plates were incubated at 42°C for 48 h. For acetic acid stress assays (Fig. 3b), serial dilutions from 10^{-1} to 10^{-3} were spotted onto SD-ura agar plates supplemented with 1 g/L acetic acid, and plates were incubated at 30℃ for 48 h.

ROS content determination: A total number of approximately 2×10^7 cells were collected using a centrifuge machine at 12000 rpm for 6 min at 4°C, and then ROS levels were assessed with DCFH-DA staining as per the protocol reported by Kim & Xue (2020). The solution of DCFH-DA (2′,7′-Dichlorodihydrofluorescein diacetate) (2 μM) in DMSO was prepared and incubated at 30°C for 15 min after the addition of the cell suspension (Kim & Xue, 2020). Cells were subsequently washed three times with PBS to remove excess dye. ROS content was determined by fluorescence analysis using a Cytation 3 microplate reader (BioTek, USA) with λ_{ex} = 488 nm and λ_{em} = 525 nm.

Statistical analysis: All quantitative data are presented as mean ± standard deviation (SD) from 3 independent biological replicates. For multiple comparisons across 8 strains, statistical significance was analyzed by one-way ANOVA followed by Tukey's HSD post-hoc test. For pairwise comparison between a single strain and the control, Student's t-test was used. Differences were considered statistically significant at *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Results and Discussion

GRX family protein characteristics: Eight GRXs were characterized from *Saccharomyces cerevisiae.* Grx1 and Grx2, representing the dithiol (classical) type; Grx3 and Grx4, which are multidomain monothiol proteins; and Grx5, a mitochondrial monothiol glutaredoxin. In addition, two further monothiol members, Grx6 and Grx7, function as membrane-associated proteins. Both Grx6 and Grx7 are integral membrane glutaredoxins, with their catalytic regions oriented toward the luminal side of early secretory pathway compartments. Grx6 is detected in both the endoplasmic reticulum and Golgi apparatus, whereas Grx7 is primarily localized to the Golgi. Their transcription is moderately upregulated by calcium, sodium, and oxidative stress, mediated by calcineurin/Crz1 signaling in Grx6 and by Msn2/Msn4 in Grx7. These regulatory patterns indicate their role in maintaining redox balance in the lumen under fluctuating environmental conditions (Izquierdo *et al*., 2008). GRX8 is a disulfide-type GRX located in the cytoplasm (Fig. S1). Glutaredoxins (GRXs) possess a catalytic motif characterized by either a Cys–X–X–Cys or Cys–X–X–Ser sequence, positioned within a flexible loop that links the first β-strand to the adjacent α-helix. Fig. 1a highlights the conserved structural features of the GRX family, including the classical Cys-X-X-Cys active site, the cis-proline motif, and the GRX-specific GG motif.

Transcriptional profiling of *GRX* genes under stress: The transcriptional heatmap in Fig. 1b shows that *Grx1* and *Grx2* exhibit the highest basal expression at 30°C. This indicates that they play an important role in maintaining redox homeostasis. Their downregulation at 40°C suggests stress-induced redistribution of redox capacity. At the same time, their reinduction under acetic acid exposure indicates an expanded role in weak-acid detoxification and glutathione-dependent redox buffering. Together, the motif architecture in Fig. 1a and the stress-responsive transcription patterns in Fig. 1b demonstrate the central roles of *Grx1* and *Grx2* in adaptive redox homeostasis and align with experimentally observed increases in thermotolerance and acetic-acid tolerance upon overexpression of these genes.

Weak-acid stress involves not only intracellular acidification but also perturbation of NADH balance, anion accumulation, and redox cycling (Demchuk & Patel, 2020).

The transcript levels of the *GRXs*, *GRX3* and *GRX4*, are minimal at 30°C, rise under heat stress at 40°C, and decline when cells are exposed to acetic acid. This pattern suggests that these genes respond more strongly to elevated temperature than to acid-induced stress. In contrast, *GRX7* and *GRX8* show comparatively higher expression at 30°C but are downregulated at 40°C and under acetic acid treatment. *GRX7*, which is linked to the endoplasmic reticulum, participates in ER stress signaling (*Izquierdo et al*., 2008).

Confirmation of *GRX* overexpression: qRT-PCR results demonstrated that *Grx* transcript levels increased by approximately 1.8- to 4.1-fold in the modified strains (Fig. S3).

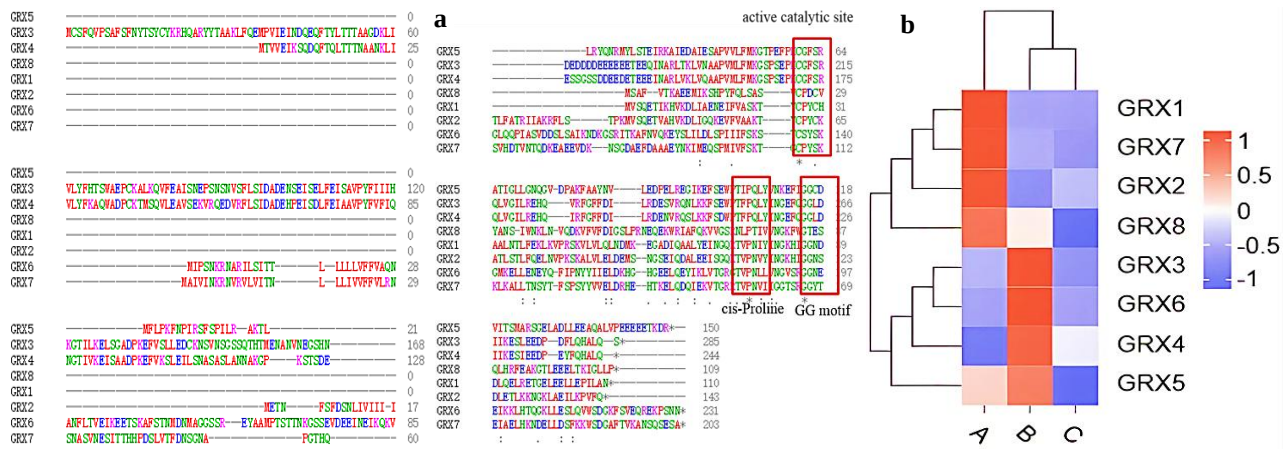

Fig. 1. Characterization of *GRX*s in Saccharomyces cerevisiae and their transcriptional profiling.
(a) Multiple sequence alignment (MSA) of all eight *GRX* isoforms (*GRX1–GRX8*). The conserved active catalytic site and cis-proline/GG motifs are highlighted in red boxes.
(b) Expression analysis of *GRX* genes under the three experimental conditions (A, B, C). Values represent normalized relative expression levels: raw FPKM values were first $\log_2$-transformed ($\log_2$ (FPKM+1)), followed by row-wise z-score normalization. The color scale bar (right) indicates normalized expression levels, ranging from-1 (blue, low expression) to 1 (red, high expression).

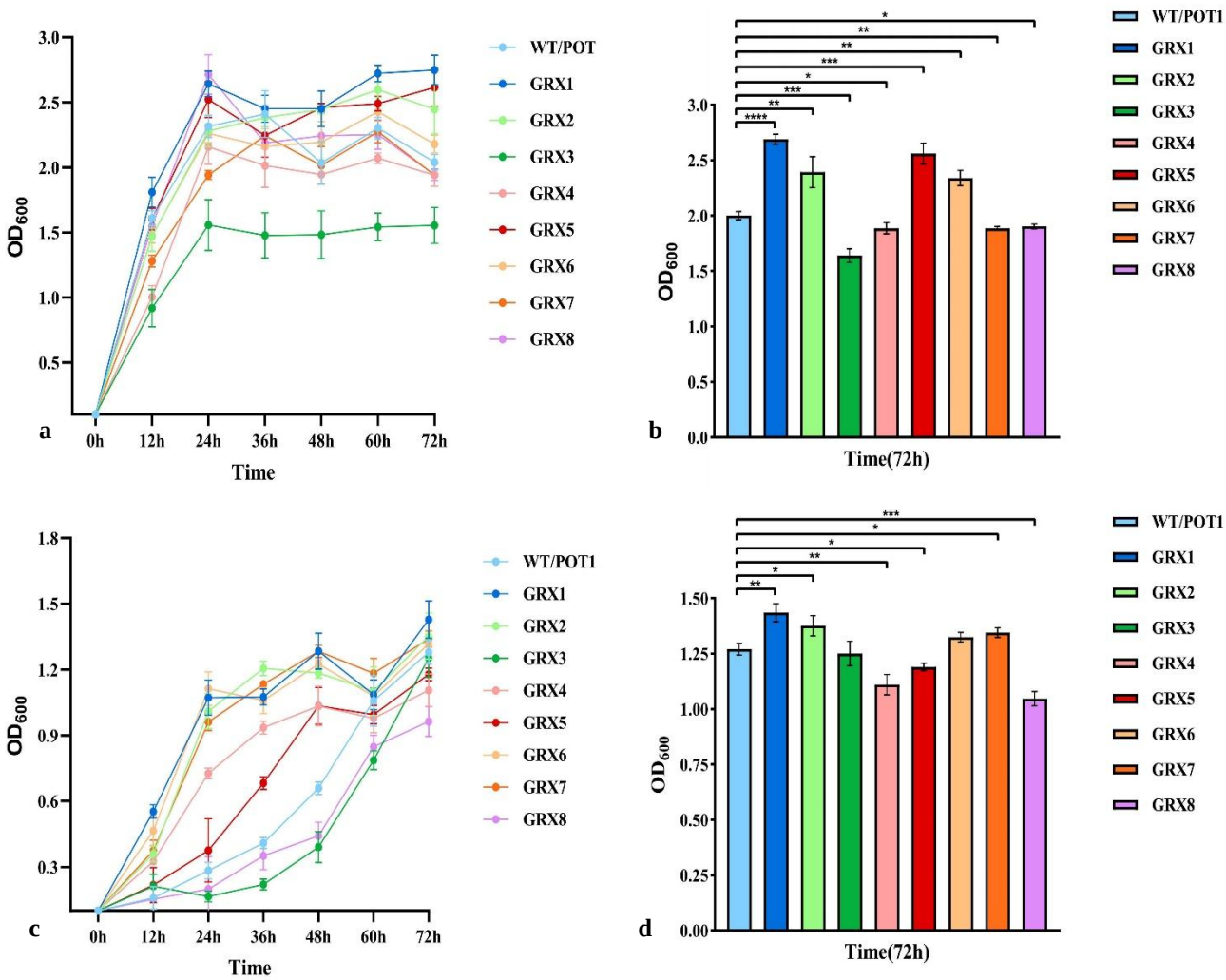

Fig. 2. Growth performance of engineered strains under stress conditions.
(a) Growth kinetics at 42℃;
(b) OD_{600} (absorbance at 600 nm) measured at 72 h under 42℃;
(c) Growth profile in the presence of 1 g/L acetic acid;
(d) OD_{600} (absorbance at 600 nm) recorded at 72 h with 1 g/L acetic acid.
Significance markers in panels (b) and (d) indicate results of one-way ANOVA followed by Tukey's HSD test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

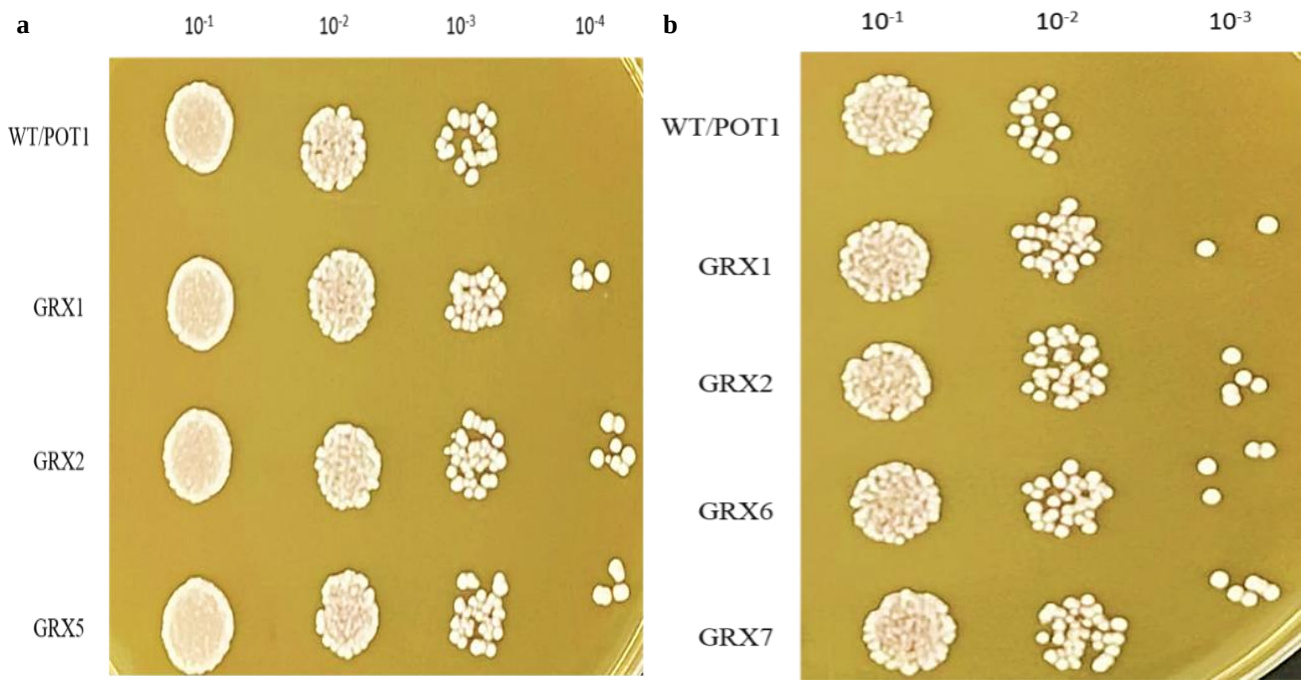

Fig. 3. Qualitative spot assay of engineered strains under stress conditions. (a) Growth performance of strains under 42℃ heat stress after 24 h of cultivation. Serial dilutions were prepared from 10^{-1} to 10^{-4}. (b) Growth performance of strains under 1 g/L acetic acid stress after 24 h of cultivation. Serial dilutions were prepared from 10^{-1} to 10^{-3}.

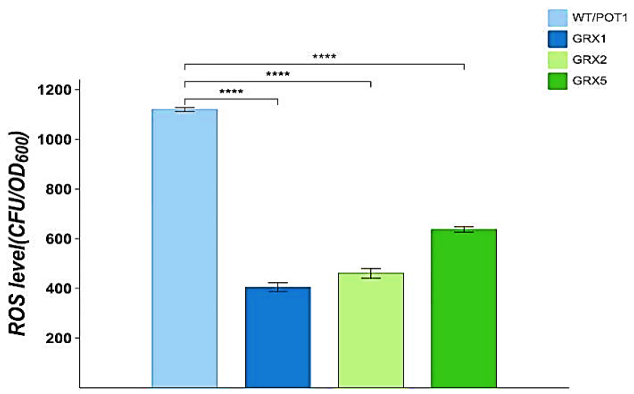

Fig. 4. Determination of intracellular ROS levels.
The Y-axis represents the absolute ROS level (CFU/OD_{600}), directly reflecting the intracellular concentration. No normalization relative to the wild-type was performed. Error bars indicate standard deviations (n = 3). Statistical significance was determined using Student's t-test, with $p<0.0001$ indicating a highly significant difference compared to the control strain (empty POT1 plasmid).

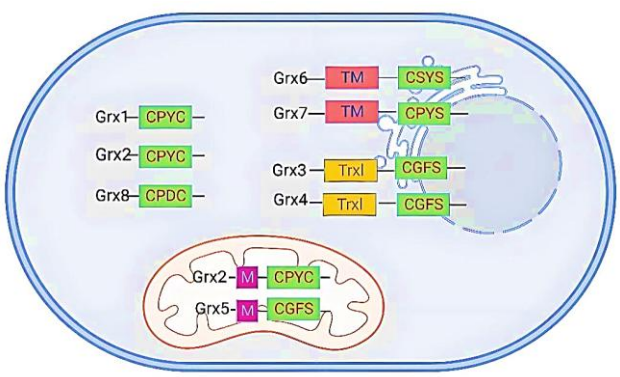

Fig. S1. *GRX* Species and their distribution.

Impact of GRX overexpression on stress tolerance: Yeast strains expressing the *Grx* expression system were subjected to physicochemical stress of 42°C and 1 g/L acetic acid. Yeast strains expressing *Grx1*-, *Grx2*-, and *Grx5* demonstrated markedly improved growth at 42°C. On the other hand, yeast strain expressing *Grx3* showed reduced growth (Fig. 2a). The strains expressing *Grx1*, *Grx2*, and *Grx5* separately exhibited increases in growth of 34.5%±0.2%, 19.6%±5.0%, and 28.0%±2.5%, respectively, compared to the control after 72 h (Fig. 2b and Fig. 3a). This magnitude of improvement suggested that cytosolic redox buffering capacity became a rate-limiting factor under combined thermal and oxidative stress. The yeast strain expressing following GRXs such as *Grx1*, *Grx2*, *Grx6*, and *Grx7* genes exhibited significantly increased growth under acetic acid stress (Fig. 2c). In contrast, the growth of the strain overexpressing the *Grx8* gene was inhibited. The ER/Golgi redox environment is tightly regulated by monothiol GRXs such as *Grx6*/*Grx7*, which modulate disulfide-bond isomerization and maintain luminal redox balance under stress. Their overexpression likely counteracts acetic-acid-induced secretory stress (Izquierdo *et al.*, 2008). Acetic acid stress is known to cause intracellular acidification, increased ROS generation, and mitochondria-associated programmed cell death. The enhanced growth observed in strains overexpressing Grx1, Grx2, Grx6, or Grx7 under 1 g/L acetic acid indicates that reinforcement of the glutaredoxin network mitigates oxidative imbalance and protects the secretory pathway from damage induced by weak acid exposure. The OD of yeast strains expressing *Grx1*, *Grx2*, *Grx6*, and *Grx7* was 13.0%±4.0%, 8.3%±1.4%, 4.3%±0.5%, and 5.9%±1.5% higher than that of the control strain (empty POT1 plasmid) strain, respectively, after 72 h of fermentation (Fig. 2c, d). Growth inhibition in the *Grx8*-overexpressing strain may result from dysregulated disulfide bond formation, leading to protein misfolding and increased cellular stress.

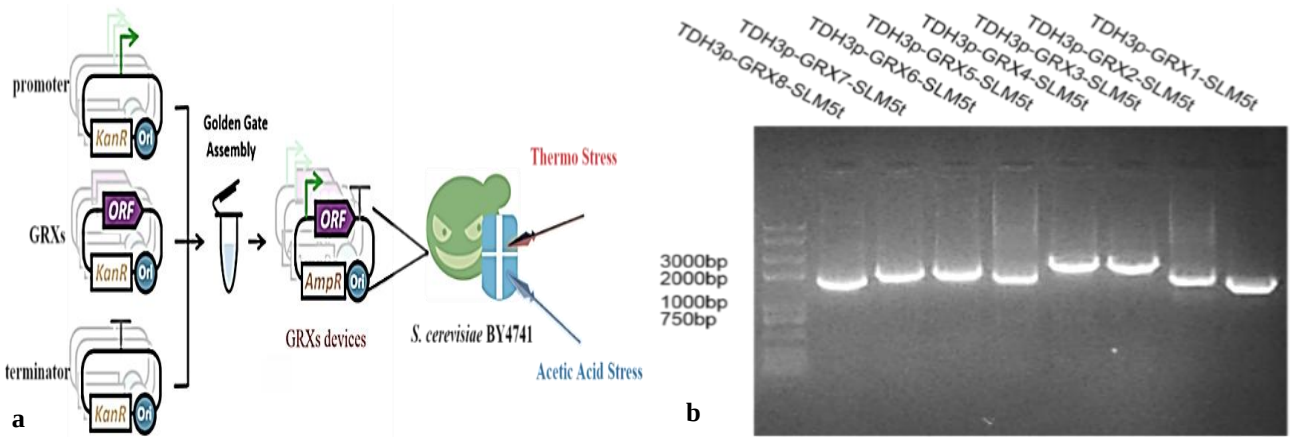

Fig. S2. Construction of *GRX* devices using Golden Gate Assembly.
(a) Overview of the construction strategy.
(b) Agarose gel electrophoresis of *GRX* devices. Lane M: DL8000 DNA marker (100, 250, 500, 750, 1000, 2000, 3000, 5000, and 8000 bp). From left to right, the expected sizes of the assembled GRX devices are 1400, 1712, 1796, 1553, 1835, 1958, 1532, and 1433 bp, respectively.

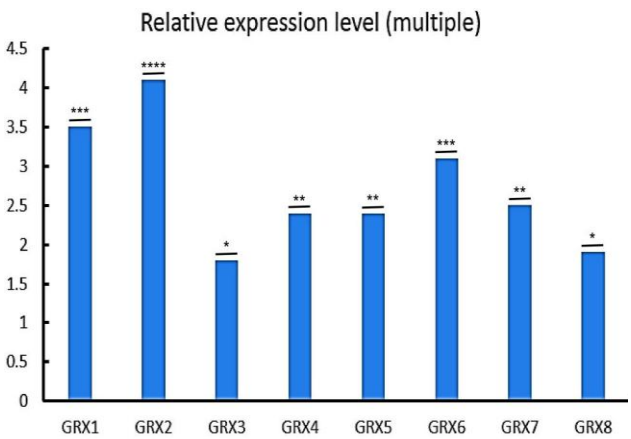

Fig. S3. Relative expression levels of *GRX* genes in recombinant strains.
The Y-axis represents relative expression levels normalized to the control strain (empty POT1 plasmid) (set to 1).

Analysis of ROS levels in yeast strains: The growth of yeast at elevated temperatures can lead to the denaturation of the protein component of the electron transport chain, resulting in ROS leakage. Exposure to mild heat stress triggers pronounced structural reorganization of mitochondria and additional cellular organelles. These changes include modifications in cristae architecture and alterations in vacuolar structure, reflecting disrupted respiratory function and increased generation of reactive oxygen species (ROS) (Giannattasio *et al*., 2013). Heat shock causes mitochondrial hyperpolarization and ROS bursts, which activate antioxidant systems such as thioredoxins and glutaredoxins (Slimen *et al*., 2014). *Grx1*, *Grx2*, and *Grx5* overexpressing strain showed enhanced growth at 42°C, which was consistent with their roles in redox regulation. *Grx1* and *Grx2* act as disulfide oxidoreductases that support glutathione-dependent detoxification. *Grx1* primarily protects against superoxide and organic hydroperoxides, whereas *Grx2* is more active against hydroperoxides. Both enzymes cooperate with glutathione transferases in peroxide and xenobiotic defense, as well as with mitochondrial *Grx2* (Herrero *et al*., 2011; Luikenhuis *et al*., 1998). Recent evidence highlights *Grx5* as a central scaffold for Fe–S cluster transfer to multiple mitochondrial client proteins; its dysfunction increases ROS sensitivity by impairing Fe–S maturation (Jang *et al*., 2021). The overexpression of *Grx1*, *Grx2*, and *Grx5* genes significantly reduced intracellular accumulation of ROS in the engineered strain compared with the control strain (empty POT1 plasmid) strain under 42°C (Fig. 4).The reduction in ROS accumulation indicates improved electron transport chain stability and enhanced glutathione recycling efficiency in the engineered strains. The ROS accumulation in strains expressing *Grx1*, *Grx2*, and *Grx5* genes was 36.2%±1.0%, 41.2%±1.7%, and 58.8%±0.8% of that in the wild-type strain. Enzymatic analyses indicate that *Grx2* provides the majority of GSH-dependent disulfide oxidoreductase activity in yeast. Loss of *Grx2* lowers this activity to approximately 15–20% of control strain (empty POT1 plasmid) levels, while simultaneous deletion of *Grx1* and *Grx2* nearly eliminates it, underscoring their dominant role in maintaining cytosolic redox balance (Luikenhuis *et al*., 1998). Our results show that overexpression of Grx6 and Grx7 enhances growth under acetic acid stress align with their proposed function in controlling disulfide homeostasis within the early secretory pathway.

Collectively, these findings indicate that strategic reinforcement of the GRX network via coordinated overexpression effectively enhances the cellular tolerance of *Saccharomyces cerevisiae* to the key redox stresses (e.g., acetic acid and heat stress) inherent in industrial fermentation processes, a strategy that aligns with the conserved role of GRXs in mediating redox homeostasis in yeast (Herrero *et al*., 2011).

Conclusion

This study demonstrates that engineering the glutaredoxin (GRX) network through targeted overexpression markedly enhances *S. cerevisiae's* resistance to key physicochemical stresses encountered during lignocellulosic bioprocessing. *Grx1*, *Grx2*, and *Grx5* significantly improved thermotolerance, whereas

Grx1, *Grx2*, *Grx6*, and *Grx7* strengthened tolerance to acetic acid, highlighting stress-specific functional roles within the GRX system. By reducing intracellular ROS accumulation and stabilizing redox homeostasis, the GRX device provides a practical synthetic biology strategy to improve yeast robustness under industrially relevant conditions. From an industrial application standpoint, reinforcing intracellular redox circuits may reduce process failure rates during high-gravity or high-temperature fermentation operations.

These findings establish glutaredoxin pathway amplification as a promising approach to enhance yeast fermentation performance.

Data available statements: All data generated or analyzed during this study are included in the manuscript and its supplementary files. Strains and plasmids are available from the corresponding author upon reasonable request.

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Author's Contribution: Yazi Li, Zhixin Zhang, Jingjie Zhang and Lin Wang designed and performed strain construction and fermentation studies and revised the manuscript. Jiaru Gao conducted transcriptomic and data analysis. Lishuang Zhang and Robina Manzoor contributed to strain engineering and fermentation validation. Meiling Zhang and Yongshan Fan assisted with fermentation experiments. Ke Xu supervised the study, secured funding, guided methodology, and revised the manuscript. All authors approved the final version.

Declaration of Competing Interest: The authors declare no competing financial or personal interests.

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