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Review Article

Engineering transcriptional regulatory networks for improving second-generation fuel ethanol production in *Saccharomyces cerevisiae*

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ABSTRACT

Presently, *Saccharomyces cerevisiae* demonstrates proficient co-fermentation of glucose and xylose, marking a significant advancement in second-generation fuel ethanol production. However, the presence of high concentrations of inhibitors in industrial lignocellulose hydrolysates and post-glucose effect caused by glucose consumption hinders severely impedes yeast robustness and xylose utilization for ethanol fermentation. Even worse, the antagonism between xylose utilization ability and strain robustness was observed, which proposes a difficult challenge in the production of second-generation fuel ethanol by *S. cerevisiae*. This review introduces the effect of engineering transcriptional regulatory networks on enhancing xylose utilization, improving strain robustness, alleviating antagonism between xylose utilization and strain robustness, and reducing post-glucose effect. Additionally, we provide an outlook on the developmental trends in this field, offering insights into future directions for increasing the production of second-generation fuel ethanol in *S. cerevisiae*.

1. Introduction

Yeast is an important chassis for research in medicine, food, cosmetics, and biofuels [1,2], among which expanding the utilization of xylose by *S. cerevisiae* is of great significance to the biofuel industry. Converting lignocellulose to ethanol for liquid fuel is called second-generation fuel ethanol. Lignocellulose hydrolysate primarily comprises glucose and xylose. While yeast can efficiently utilize glucose as the primary carbon source, their ability metabolized xylose is limited. In recent studies, *S. cerevisiae* has emerged as a promising chassis cell, demonstrating co-fermentation capacibility for glucose/xylose conversion into ethanol. This has been achieved by introducing xylose isomerase (XI) and improving its expression activity, optimizing endogenous sugar metabolism pathways, and introducing xylose-specific transporters [3]. When xylose was used as the sole carbon source of engineering strains, Chen and Demdke et al. reported specific xylose utilization rates of 0.851 and 1.10 g g^{-1} h^{-1}, with corresponding ethanol yields of 0.437 and 0.46 g g^{-1}, respectively [4,5]. Additionally, our engineered strain achieved an impressive specific xylose utilization rate of 1.09 g g^{-1} h^{-1} and an ethanol yield of 0.446 g g^{-1} [6]. However, lignocellulosic hydrolysates generally contain toxic factors with high concentrations, which severely impaired the yeast's xylose utilization capacity (Fig. 1a).

Ding et al.'s study showed leachate without detoxicification treatment and the presence of high concentration ethanol significantly inhibited xylose metabolism, respectively. The strain demonstrated complete metabolism of 60 g L^{-1} xylose within 24 h of fermentation under inhibitor-free conditions, however, when exposed to

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lignocellulosic hydrolysate or high concentration ethanol condition, xylose levels remained at 38 g L^{-1} or 52.2 g L^{-1}, after 96 h of fermentation. Additionally, ethanol yield decreased from 0.447 g g^{-1} to 0.392 g g^{-1} and 0.307 g g^{-1}, respectively [7]. Zhang et al. found that 100 g L^{-1} glucose and 50 g L^{-1} xylose can be completely utilized in an inhibitor-free mixed sugar medium for 72 h fermentation. However, xylose remained at 25 g L^{-1} when the fermentation was conducted in real rice straw hydrolysate with an equivalent sugar concentration after 144 h [8]. These studies underscore the strain's sensitivity to hydrolysate toxicity or stress when metabolizing xylose. Numberous studies attribute the hindrance in xylose metabolism within hydrolysates to the strain's inadequate resistance to inhibitors. Consequently, extensive research has focused on improving the strain's robustness to increase inhibitor tolerance [9,10]. Interestingly, researchers have observed an inverse relationship between improved robustness and xylose utilization ability [6,11]. Our research also identifi this issues, which we termed the "antagonism between xylose utilization ability and robustness" [12].

Most studies have primarily examined the xylose metabolism ability of strains in medium where xylose is the sole carbon source, with less emphasis on understanding xylose metabolism characteristics using mixed sugars containing both glucose and xylose as the carbon source (Fig. 1b). Wei et al. conducted research on haploid yeast BSG001 and diploid yeast XH7, both featuring similar genetic modifications. In medium using xylose as the sole carbon source (named X), the specific xylose utilization rates were 0.461 and 0.747 g g^{-1} h^{-1}, respectively. However, In the mixed sugar medium where xylose can be used as the carbon source only after complete consumption of glucose (named GX), the specific xylose utilization rates decreased by 30.0 % and 58.6 %, respectively [13]. In a recent study by Chen et al., four engineered strains can metabolize xylose and exhibited comparable specific xylose utilization rates in the X: 0.851, 0.832, 0.698 and 0.808 g g^{-1} h^{-1}, respectively [5]. Yet, in the GX, specific xylose utilization rates of the strains decreased by 50.88 %, 40.63 %, 72.64 % and 62.25 % respectively. This phenomenon, termed the "post-glucose effect" on xylose metabolism [13], was not influenced by ethanol production [14]. Numerous studies on co-fermentation of glucose/xylose have highlighted the presence of post-glucose effect across strains with different genetic backgrounds, albeit with varying intensities.

Recent investigations have identified, a series of related transcription factors (TFs) and functional genes based on the study of antagonism and post-glucose effect [15]. Transcriptional regulation mediated by these TFs influences various aspects of cell function, potentially impacting the xylose metabolism pathway and strain robustness and growth. These effects can be both positive and negative. Elucidating the mechanism underlying their regulations holds substantial importance in guiding relevant engineering strategies. This article systematacially introduces the effect of engineering transcriptional regualatory networks on enhancing xylose utilization, improving strain robustness, alleviating antagonism between xylose utilization ability and strain robustness, and reducing post-glucose effect.

2. Engineering transcription factor regulatory network improves the robustness of *S. cerevisiae*

Yeast must navigate various stresses during lignocellulosic hydrolysate fermentation, including weak acids, furan aldehyde, phenolic compounds and unidentified trace substances in lignocellulose hydrolysate. Additionally, acidic substances and ethanol produced during fermentation process also add to this stress [7,16]. Acetic acid, a representative weak acid, exerts toxicity by interfering the intracellular pH stability and resulting in metabolic pressure [17,18]. Furan aldehydes, such as furfural and 5-hydroxymethylfurfural (5-HMF), primarily induce toxicity through oxidative stress and DNA damage caused by their aldehyde groups [19,20]. Phenolic compounds, recognized as the most toxic inhibitors in hydrolysates, contain benzene rings, methoxy groups, hydroxyl groups, carboxyl groups, and aldehyde groups. They induce membrane damage, oxidative stress, and inhibit ribosome biogenesis [21,22]. The toxicity of ethanol manifests in membrane integrity disruption and the inhibition of key enzyme activity in the glycolysis (EMP) pathway [23]. The combination of these stressors result in stronger synergistic toxicity, intensifying stress on yeast [24,25]. Under stress conditions, alterations occur in yeast cell wall/-membrane composition, oxidoreductase activity, energy and coenzyme supply, and amino acid and ribosome synthesis characteristics [20]. These changes are regulated by TFs such as Hog1p, Msn2/4p, Haa1p, Yrr1p, Yap1p, Stb5p and Ecm22p (Fig. 2, Table 1). Hog1p primarily targets genes associated with osmotic pressure and oxidative stress, including *MSN2/4*, *YAP1*, and *HAA1*. Msn2/4p, Yap1p, and Stb5p are all involved in oxidative stress responses. Haa1p is a transcription factor that responds to weak acid stress, while Yrr1p is uniquely active in the presence of the phenolic compound vanillin. Ecm22p plays a crucial role in maintaining cell membrane integrity. These factors are simultaneously activated in lignocellulosic hydrolysate environments, indicating potential synergistic roles in improving robustness [26].

2.1. *Strengthening strain robustness by regulation of osmotic pressure-related transcription factor Hog1p*

The transcription factor Hog1p is vital for resistance to hydrolysate toxicity, and *HOG1Δ* mutant strain exhibits poor growth in a hydrolysate environment [39]. Hog1p is a component of the HOG-MAPK pathway, comprising two Hog1p activiating branches, namely Sln1 and Sho1 branches [40]. In a mild environment, Sln1p undergoes phosphorylation and transfers the phosphate group to Ypd1p and Ssk1p. However, phosphorylated Ssk1p fails to interact downstream Ssk2p and Ssk22p, leading to signal interruption. Under stress conditions, Sln1p undergoes

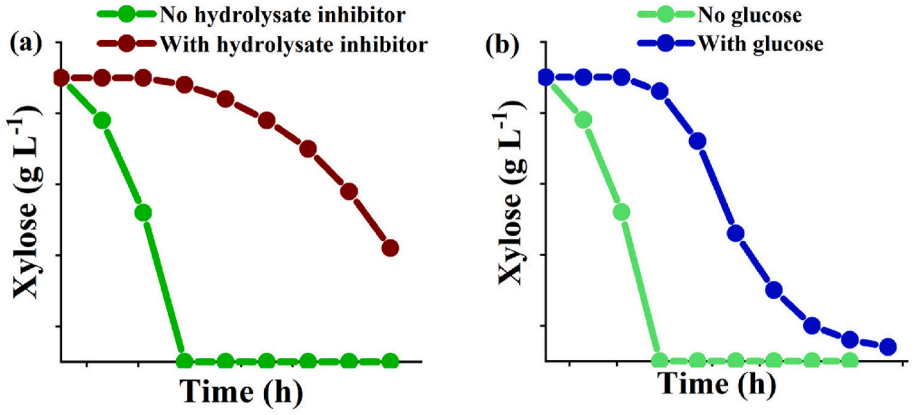

Fig. 1. Xylose metabolism characteristics. Hydrolysate inhibitors impair xylose consumption (a). Glucose inhibits xylose consumption (b).

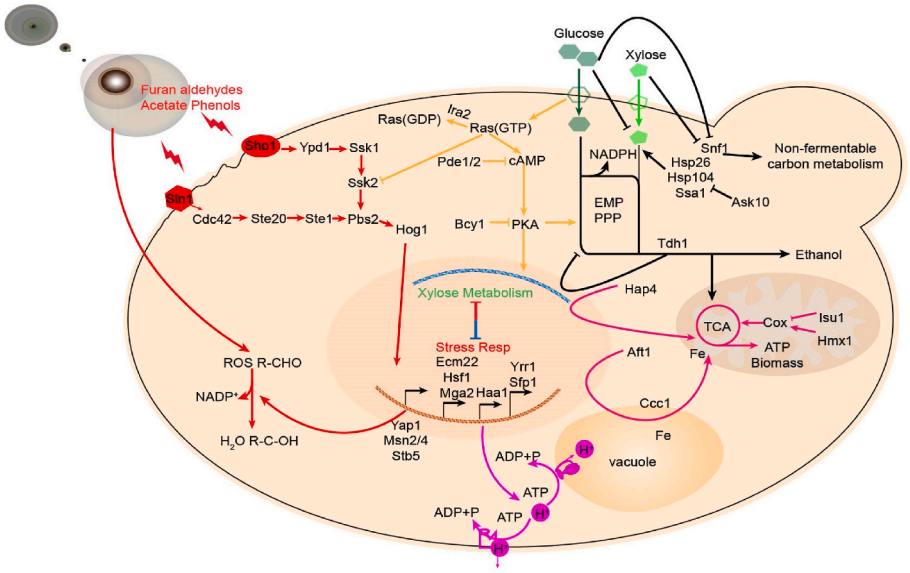

Fig. 2. The antagonism between xylose metabolism and robustness of yeast in lignocellulosic hydrolysate.

Table 1
Enhancing robust related TFs.

TFs	Target gene function	Reference
Hog1p	Osmotic and oxidative stress	[27]
Msn2/4p	Heat shock and oxidative stress	[28]
Yap1p	Oxidative stress and coenzyme supply	[29]
Haa1p	H^+ efflux	[30]
Stb5p	Oxidative stress and coenzyme supply	[31]
Ecm22p	Ergosterol synthesis	[32]
Upc2p	Ergosterol synthesis	[32]
War1p	H^+ efflux	[33]
Mig1p	Non-fermentable carbon sources metabolism	[34]
Hsf1p	Heat shock and H^+ absorption	[35]
Yrr1p	Multidrug resistance	[36]
Sfp1p	Ribosome biogenesis and mitosis	[37]
Ume6p	Ribosome biogenesis and mitosis	[38]

dephosphorylation, and nonphosphorylated Ssk1p binds to Ssk2p, resulting in the phosphorylation of Ssk2p. This in turn transfers the phosphate group to the scaffold protein Pbs2p. And Sho1p directly interacts with Msb2p/Hkr1p, activating the membrane-bound GTPase Cdc42p. Cdc42p then transmits signals to the scaffold protein Pbs2p via Ste20p, Ste50p and Ste11p [41]. Phosphorylated Pbs2p further phosphorylates Thr174 and Tyr176 in the T-G-Y motif of Hog1p, activating Hog1p [42]. Activated Hog1p translocates into the nucleus facilitated by Gsp1p and Nmd5p, where it interacts with chromatin modification enzymes and RNA polymerase II. This interaction affects the expression of numberous genes, including other TFs related to stress resistance, such as Msn2/4p and Sko1p [43]. Researches indicate that the phosphorylation level of Hog1p correlates with the strength of strain robustness [44,45].

Regulating the phosphate signal intensity of the HOG-MAPK pathway can effectively increase Hog1p activity. Takayama et al. found that $OPY2^{A104V}$, a regulatory protein in the HOG-MAPK pathway, enhanced its binding activity to $STE11^{Q301P}$. This led to increased phosphorylation levels of Pbs2p by Ste11p, ultimately activating Hog1p significantly [27]. Chen et al. observed that the addition of zinc sulfate to an acetic acid environment significantly increased Cdc42p transcription levels. Strain overexpressing *CDC42* were found to confer resistance to various stresses [46]. Nasution et al. demonstrated that overexpression of fatty acid desaturase Ole1p constitutively activated Hog1p, improving proton efflux and reactive oxygen species (ROS) scavenging capabilities. Further analysis revealed the involvement of Ssk2p activation in this process [47]. Activated Hog1p mediates changes in nearly 600 genes by phosphorylating TFs [43,46,48]. Functional genes directly or indirectly regulated by Hog1p include *ZWF1*, *CTT1*, *SOD1*, *GRE2*, *OLE1*, *CCW12*, *FPS1*, etc [49], affecting redox reactions, coenzyme supply, changes in cell membrane/wall composition, proton efflux and other cellular processes. Therefore, these reported genes could be used as engineering targets to regulate the HOG-MAPK pathway to improve strain robustness during hydrolysate fermentation.

2.2. Regulation of strain robustness to oxidative stress by TFs Msn2/4p, Yap1p and Stb5p

Msn2p and Msn4p are homologous TFs in responding to stress in *S. cerevisiae*. Various stressors, such as acetic acid, ethanol, furan aldehyde, and ROS, activate the transcriptional regulation of *MSN2/4*. The regulation of *MSN2/4* extends beyond the HOG-MAPK pathway to include the Ras-PKA pathway [50]. PKA pathway inactivite under stress conditions, leading to the activation of Ssk2p, which activate Msn2/4p via Hog1p. During this period, Msn2/4p undergoes dephosphorylation and promptly translocates into the nucleus for transcriptional regulation [51]. Under sustained stress, Msn2/4p periodically enters the nucleus [52]. While deletion of *MSN2* or *MSN4* alone does not result in obvious phenotypic changes, simultaneous deletion of both genes renders cells sensitive to various stresses [53]. Studies have shown that overexpression of fragmented *MSN2* alters its transcriptional activity, consequently increasing ethanol tolerance [54,55]. Specifically, the deletion of the first 50 amino acids in the transcriptional activation region of Msn2p eliminates its interaction with Gal1p, leading to a slower degradation of Msn2p [56]. The promoters of Msn2p targets genes contain stress response elements (STREs) AGGGG or CCCCT, with most also containing G4 DNA [57]. These target genes are primarily associated with stress response and heat shock response [58], including the regulation of ROS-related genes such as *CTT1*, *SOD1*, *PRX1* and *TSA2* [59,60]. Additionally, these genes are subjected to regulation by other TFs such as Yap1p and Stb5p. Qin et al. demonstated that enhancing the expression of the pentose phosphate pathway (PPP) oxidation stage and key enzymes in the glutathione synthesis pathway improved regeneration of reduced coenzymes and reduced ROS accumulation [61]. Du et al. found that Msn2p targeted the heat shock transcription factor *HSF1*, subsequently regulating the expression of heat shock-related genes, including *HSP104*, *HSP78*, *HSP82* and *HSP12* [28], impacting yeast toxicity and heat tolerance. Moreover, Msn2p is implicated in

amino acid homeostasis, and overexpression of *MSN2* proves beneficial in increasing the expression level of proline permease, thereby increasing cell absorption of the stress-protective proline [62].

To alleviate the inhibitory effects of hydrolysates, substantial amounts of reducing coenzymes, especially NADPH, are required. The PPP pathway serves as a crucial source of coenzyme NADPH, and enhancing its flux is important for yeast to withstand oxidative stress. The activation of TFs, such as Yap1p and Stb5p, positively influences on the PPP pathway. However, there exists a difference between their effectiveness. Stb5p exhibits superior activation of the PPP pathway compared to Yap1p, resulting in a more potent detoxification effect on furfural [29]. On the other hand, Yap1 proves more adept at improving resistance to coniferyl aldehyde due to the involvement of target genes FLR1 and ATR1 in coniferyl aldehyde degradation [63]. Additionally, enzymes encoded by genes like *ADH6* and *ADH7*, *YNL134C*, *AAD4*, *AAD14* and *YJR096W* demonstrate activity against phenolics [64]. Research indicates that overexpression of *STB5* generates sufficient NADPH for free fatty acid synthesis [31]. Yap1p and Stb5p may also influence the purine synthesis pathway, as the initial purine synthesis materials derive from 5-phosphate-ribose produced by the PPP pathway. Overexpression of *ADE1*, *ADE13* and *ADE17* which involved in de novo purine synthesis has been shown to improve strain growth under various stresses [65], suggesting a potential link to increased ATP synthesis and the production of the stress protectant gamma-aminobutyric acid which caused by purine synthesis. These results demonstates engineering TFs involved in oxidative stress resgulation is a promising way to improve strain robustness against hydrolysates.

2.3. Regulation of strain robustness to ethanol and phenols by TFs Ecm22p, Hsf1p and Mga2p

A robust yeast response to hydrolysate stress involves an integrated cell wall structure, high trehalose and ergosterol content. Higher ergosterol content enhances yeast tolerance to phenolic compounds [66]. TFs *ECM22* and *UPC2* regulate ergosterol synthesis at low sterol levels [32], upregulating the transcription of *ERG1*, *ERG2*, *ERG3*, *ERG25*, *ERG26*, and *ERG27*. Trehalose synthesis-related genes *TPS1* and *ATH1* are regulated by TFs Msn2/4p and heat shock-associated transcription factor Hsf1p and high trehalose content provides better resistance to high concentrations of ethanol [35,67]. A robust cell wall ensures the normal function of membrane proteins and intracellular homeostasis. Overexpression of *CCW12* improved yeast resistance to corn hydrolysate, miscanthus hydrolysate and corncob hydrolysate, with the cell wall thickness of the overexpressing strain (282.9 nm) significantly higher under acetic acid stress compared to the control strain (109.8 nm) [68]. Cell membranes rich in unsaturated fatty acids have higher fluidity, leading to a positive effect on weak acid stress. Fatty acid desaturase *OLE1* is significantly upregulated under oxidative stress [69]. It is mainly regulated by the endoplasmic reticulum protein Mga2p [70]. Expression optimization of TFs to control cell wall structure, ergosterol content and trehalose content may be an attractive method to increase strain robustness.

2.4. Regulation of strain robustness to weak acid by TFs Haa1p and Mig1p

The transcription factor Haa1p exhibits a sophisticated response mechanism to weak acid stress. Yeast Haa1p regulates approximately 80 % of acid tolerance-related genes, making it a promising avenue for improving weak acid resistance [71]. Haa1p target genes encompass a wide array of functions, invoving in carbohydrate metabolism, multidrug resistance, lipid and amino acid metabolism, cell wall biosynthesis, protein folding, and nucleic acid modification [30,72]. The toxicity of weak acids is closely related to pH [16]. Under acetic acid stress, yeast cells overexpressing *HAA1* demonstrated lower acetic acid content due to the mediation of H^+ efflux by its target gene [73]. Transcriptome analysis showed that the upregulation of Haa1p target genes *TPO2/3*, encoding membrane proteins involved in the export of polyamines, *YRO2* encoding a plasma membrane protein, and *YGP1*, encoding cell wall-related secretory glycoprotein, were play essential roles in response to acid stress [74]. Vacuoles are pivotal for maintaining cellular homeostasis. The structure of vacuole membrane contains V-ATPase, which pumps H^+ from the cytoplasm into the vacuole, together with Haa1p-targeted H^+ efflux proteins in cell membranes, maintaining intracellular pH homeostasis [75]. In addition, the transcription factor War1p is also involved in the response to weak acid stress, mainly by regulating the ABC transporter Pdr12p [33].

When H^+ in hydrolysate penetrates the cell, it often leads to an increase in intracellular acetate concentration. Under high glucose conditions, the non-phosphorylated transcription factor Mig1p interacts with the inhibitory factor Cyc8/Ssn6-Tup1, and then binds to promoter of genes related to nonfermentative carbon source utilization, leading to gene expression inhibition and non-utilization of acetate [34]. *MIG1* deletion enhances tolerance to weak acids in hydrolysate, facilitating simultaneous utilization of glucose and weak acids. Following *MIG1* disruption, the expression of *FDH1* and *ACS1* was induced to utilize weak acids such as formic and acetic acid [76]. Notably, down-regulated expression of genes related to oligosaccharide metabolism benefits yeast robustness. For example, the maximum specific growth rates of *MAL33Δ* strain and *GAL3Δ* strain under acetic acid conditions increased by 3.5 times and 4.2 times respectively (data not shown). While the expression of these genes is all regulated by Mig1p, the specific mechanism requires further study.

2.5. Regulation of tolerance to vanillin and other inhibitors by TFs Yrr1p and Sfp1p

Zn(II)2Cys6 zinc finger transcription factor play a crucial role in regulating the response to external stress as well as the synthesis of amino acids and lipids [77]. Examples of these factors include Adr1p, Upc2p, Pdr1/3p and Yrr1p, which are known to be associated with bacterial resistance [78,79]. Generally, their heightened activity positively influences drug tolerance. However, our study revealed an exception, where the highly active Yrr1p had a negative impact on vanillin resistance in hydrolysate. The deletion of *YRR1* significantly improved vanillin resistance, with BY4741 (*YRR1Δ*) exhibiting a 142 % increase in the maximum specific growth rate increased and a 51 % increase in vanillin degradation rate at 6 mM vanillin, compared to the parent strain [36]. Transcript level analysis indicated that significant upregulation of genes related to alcohol dehydrogenase *ADH7*, several ABC transporters, and ribosome biogenesis. Additionally, the coenzyme preference of BY4741 (*YRR1Δ*) shifted, with a 95 % increase in NADPH preference. To validate target gene discovery, overexpression of ABC transporters *PDR5*, *YOR1* and *SNQ2* was performed, resulting in a shortened growth lag period and accelerated growth under vanillin stress [36]. Proteomic analysis revealed increased protein levels of the transcription factor Haa1p, the proteasome assembly partner Tma17p, and the transcriptional coactivator Mbf1p in the *YRR1Δ* mutant strain. Overexpressing these factors separately or simultaneously significantly improved vanillin tolerance [80]. Our study also found that, in the absence of stress, dephosphorylation mutants $YRR1^{Y134A}$ and $YRR1^{T185A}$ localized Yrr1p to the nucleus, continuously activating *SNQ2* and *YOR1*. Conversely, phosphorylation mutants $YRR1^{Y134E}$ and $YRR1^{T185E}$, while stably localized in the nucleus, suppressed target gene expression [81].

Ribosome biogenesis is linked to yeast robustness, with decreased expression of ribosome biosynthesis genes observed in highly resistant yeast [82]. Our finding aligns with this observation, suggesting that under stress conditions, yeast may reduce ribosome synthesis, increase ATP or coenzyme synthesis, and enhance stress tolerance. Overexpression of the Yrr1p-targeted ribosome synthesis-related gene *DBP2* improved vanillin tolerance. This improvement is attributed to vanillin inhibiting the synthesis of the 60S large ribosome subunit [83], and

Dbp2p's involvement in the synthesis of the 60S large subunit [84]. Wang et al. also found that formic acid significantly inhibits the synthesis of the 60S large ribosome subunit [64], implying that deletion of *YRR1* or overexpression of *DBP2* may promote strain tolerance to formic acid. *DBP2* is regulated by the transcription factor Sfp1p, a transcriptional activator of various ribosome synthesis genes in the Snf1p/Mig1p signaling pathway, jointly regulated by TORC1 and PKA. When the two proteins are activated, Sfp1p is localized in the nucleus to exert regulatory functions [37]. Chen et al. demonstrated that overexpression of *SFP1* improved yeast tolerance to hydrolysate and fermentation efficiency [26], likely due to accelerated central carbon metabolism and ribosome biogenesis. The study of *HYP1*, which promotes the synthesis of proline-containing extended-chain proteins, showed that overexpression of *HYP1* improved yeast tolerance to acetate. This improvement is associated with increased expression of the polyproline transcription factor Ume6p and its target genes *BUD21*, *IME4* and *BEM4*, which promote ribosome biogenesis, cell division and mRNA methylation modification [38].

3. TFs regulatory network improves xylose utilization in *S. cerevisiae*

Engineered strains with high xylose utilization capabilities have employed multiple strategies, including the introduction of a heterologous XI with elevated enzymatic activity, a moderate flux enhancement of PPP pathway, reduction of by-product xylitol and minimization of energy consumption, as well as the expression of xylose-specific transporters [6,85]. Recent comprehensive analyses of yeast xylose metabolism revealed significant variations in the expression of transcription factors (TFs) such as Hog1p, Ask10p, Gcr2p, and Azf1p. Ask10p, as a regulator of the aquaglyceroporin (Fps1p) glycerol channel, is required for glycerol efflux and also plays a role as a component of the RNA polymerase II holoenzyme, necessary for the degradation of Ssn8p in response to oxidative stress. Gcr2p acts as a transcriptional activator of genes involved in glycolysis, while Azf1p regulates the transcription of genes related to carbon metabolism and energy production in the presence of glucose. These TFs contribute to improved xylose metabolism in strains by mitigating the inhibitory effects on the PPP pathway, promoting proper folding of XI, enhancing PPP flux, boosting respiration, and regulating sugar signaling pathways, ultimately increasing xylose utilization (Fig. 2, Table 2).

3.1. Transcription factor Hog1p regulates xylose utilization ability in *S. cerevisiae*

The PPP pathway, the primary pathway of xylose metabolism, is essential for cell growth. Regulating TFs to mitigate inhibitors' effects on the PPP pathway can facilitate efficient xylose fermentation. A well-documented example is the enhanced xylose metabolism achieved by knocking out *HOG1*. Attenuating the phosphorylation signal of the HOG-MAPK pathway proves effective in reducing Hog1p activity. Individually knocking out *SSK2*, *SSK22* and *STE11* in the HOG-MAPK pathway doesn't disrupt Hog1p function. However, simultaneous knockout of all three genes has a detrimental effect on Hog1p activity. The absence of the scaffold protein Pbs2p has effects on yeast similar to *HOG1Δ* that leads to severely reduced cell robustness [42,48], making yeast almost unrobust [48]. Dos Santos et al.'s research found that the deletion of *SSK2* significantly improves yeast xylose utilization ability [92], indicating suppressed Hog1p activity. The study of the phosphoric acid signaling of Pbs2p and Hog1p revealed that the co-connection domain (CD) and the Pbs2p binding domain (PBD-2) are crucial for Hog1p binding to Pbs2p. Deleting CD or PBD-2 alone does not affect stress regulation by Hog1p, but simultaneous deletion renders Hog1p unable to phosphorylate [93].

Table 2
Regulate TFs to promote xylose metabolism.

Up/down TFs	Effect	Reference
Down Hog1p	Reduce interference to the PPP.	[86]
Down Ask10p	Promotes xylose isomerase folding.	[87]
Down Tdh1p	Promote central carbon metabolism.	[88]
Down Gcr2p	Promote PPP.	[89]
Up Znf1p	Promote xylose transport and reduce ribosome synthesis.	[90]
Up Azf1p	Promote anaerobic fermentation and xylose transport.	[91]
Up Mga2p	Promote anaerobic fermentation.	[91]

In our results, removing either CD or PBD-2 domains similarly reduced Hog1p activity, resulting in a decrease in robustness. However, yeast exhibited an increased ability for xylose metabolism to produce ethanol (data not shown). This phenomenon prompted us to explore the optimization of Hog1p activity levels to regulate the xylose utilization ability and strain robustness. Mutation optimization of the Hog1p domain revealed that $HOG1^{P237G}$ and $HOG1^{320-350\Delta}$ could improve the ethanol production from xylose while maintaining yeast robustness. The mutants $HOG1^{K52Y}$, $HOG1^{23-302\Delta}$ and $HOG1^{302-316\Delta}$ reduced yeast robustness, but improved the utilization capacity of xylose. Applying $HOG1^{P237G,\ 302\text{-}316\Delta}$ to the industrial strain 6M − 15 reduced Hog1p activity and increased the ethanol production from simulated hydrolysate. However, single mutants $HOG1^{302-316\Delta}$ and $HOG1^{P237G}$ demonstrated an improvement in strain robustness and a reduction in the xylose utilization ability (data not shown). This could be attributed to strain 6M-15's Hog1p being in a state of lower activity than the mutant Hog1p from the haploid strain.

Most Hog1p target genes are associated with stress, and some of them have been identified to positively impact xylose metabolism. We studied the transcriptome of strains including LF1, LF1-6M and 6M − 15 with varying degrees of antagonism between strain robustness and xylose utilization capacity, and discovered that Hog1p target genes in 6M–15 were generally at low expression levels, such as *ASK10*, *TDH1*, *FPS1* and phosphorylation-related genes in the HOG-MAPK pathways. Subsequent verification indicated that the deletion of 3-phosphoglycerate dehydrogenase *TDH1* significantly improved both the xylose utilization capacity and robustness of strains (data not shown). *TDH1*, known for its positive impact on gluconeogenesis [88], might contribute to the enhancement of central carbon metabolism upon its deletion. Similarly, the deletion of a transcription factor *CAT8* has been reported to promote xylose metabolism [94]. Wei et al. reported an enhancement in the xylose-to-ethanol conversion rate in the *FPS1Δ* mutant, suppressing xylitol production, a by-product, and reducing NADPH consumption [95]. Xiong et al.'s study demonstrated deletion of *FPS1* and *GPD2*, coupled with the overexpression of *GLN1*, effectively improved the xylose utilization of the strain, resulting in a 97 % xylose utilization rate compared to 65 % in the control strain when fermented in a synthetic medium of 50 g L^{-1} glucose and 50 g L^{-1} xylose for 48 h [96].

Hou et al. conducted a study on the transcription factor $ASK10^{M475R}$ mutant and the deletion of *ASK10*, revealing improved xylose metabolism in respiration-deficient xylose-utilizing yeast through reverse metabolic engineering. Their analysis indicated increased xylose isomerase XI activity, attributed to the elevated XI copy numbers. Further investigation found significant transcriptional upregulation of molecular chaperone *HSP26*, *SSA1* and *HSP104* in $ASK10^{M475R}$ and *ASK10Δ* strains showed, promoting the protein folding of XI [87].

3.2. TFs regulates the PPP pathway and respiration to improve xylose utilization

The distribution of carbon flux between glycolysis and the PPP is a key bottleneck in xylose metabolism. Dynamic regulation of these pathways through the activation or repression of transcription factors can significantly enhance xylose utilization. The deletion of the

phosphatase gene *PHO13* in most engineered strains has been undertaken, as it facilitates the expression of non-oxidative PPP pathway genes. Shin et al. uncovered a connection between *PHO13* and the transcription factor *GCR2*. Deletion of either *GCR2* or *PHO13* improves xylose utilization, with *GCR2Δ* reducing glycolysis and promoting the oxidative and non-oxidative stages of PPP, while *PHO13Δ* enhances glycolysis and only promotes the expression of genes involved in non-oxidative PPP pathway. Simultaneous deletion of the two genes, however, does not lead to an increase in xylose utilization [89]. Furthermore, Usher et al. reported that the deletion of the ribosomal synthesis gene *BUD21* boosted xylose utilization [97]. It is speculated that this deletion decreases the production of non-essential substances, consequently minimizing the competition with the PPP pathway, possibly related to the negative regulation of the Ras-cAMP pathway. The expression of *BUD21* is controlled by transcription factor Znf1p, involved in the regulation of glycolysis for ethanol production and yeast osmotic tolerance [98]. Songdech et al. found that overexpression of *ZNF1* positively affects xylose usage, leading to further enhancements in the *BUD21Δ* strain [90]. Analysis indicates that overexpression of *ZNF1* enhances xylose transport [90].

Additionally, optimizing respiration regulation plays a vital role in further enhancing xylose metabolism [16]. Palermo et al. investigated metal ion transporter genes, revealing that deletion of the Fe–S cluster scaffold protein *ISU1*, vacuolar Fe^{2+}/Mn^{2+} transporter *CCC1* or heavy metal homeostasis protein *BSD2* significantly improved yeast xylose metabolism. Notably, the xylose-specific consumption rate of the *ISU1Δ* strain increased by 142 % compared to the control strain [99]. In Dos Santos et al.'s study, simultaneous deletions of *ISU1* and *SSK2* achieved better results than the deletion of *ISU1* alone [92]. Osiro et al. discovered *ISU1* deletion transformed yeast sugar sensing from a low-sugar to a high-sugar signal, thereby enhancing xylose metabolism rate [86]. Palermo et al. 's analysis showed that the deletion of *ISU1*, *SSK2*, *BUD2* or *CCC1* led to a significant up-regulation of genes involved in the glycolysis and PPP pathways during the early stages of fermentation. Simutaneously, gene transcription related to respiratory chain, TCA and glyoxylate cycle was significantly down-regulated [99], consistent with changes in sugar signaling. Sato et al.' s study revealed that the deletion of *HOG1* and *ISU1* enhanced aerobic fermentation of xylose [100]. The loss of Isu1p function is speculated to impair Fe–S cluster synthesis, increase iron availability in heme biosynthesis, and promote the formation of cytochrome oxidase (COX), resulting in increased aerobic respiration using xylose as carbon source. Additionally, the deletion of *IUS1* facilitates anaerobic fermentation of xylose [100,101]. In addition, our research group found that overexpression of the heme oxygenase *HXM1* increased the metabolic rate of xylose (data not shown). *CCC1* is regulated by iron sensing [102]. *ISU1, CCC1* and *HXM1* are all regulated by the iron balance transcription factor Aft1p, suggesting that Aft1/2p plays an important role in regulating xylose metabolism.

3.3. Transcription factor regulate xylose metabolism through cAMP-PKA signaling pathway

The cAMP-PKA pathway regulates cell growth and differentiation in response to nutrients, such as glucose and xylose, by phosphorylating TFs. Dihazi et al. demonstrated that phosphorylation of cytoplasmic target genes activates glycolysis [103]. Nguyen et al. observed a close relationship between cAMP concentration and sugar metabolic rate. They successfully regulated the 8-bromo-cAMP concentration to control sugar metabolic rate in cAMP-deficient strains [104]. In the cAMP-PKA pathway, Wu et al. reported that deletion of the phosphodiesterase genes *PDE1* and *PDE2*, responsible for cAMP degradation, significantly increased cAMP concentration and trehalose content, closely linked to PKA activity. The *PDE1ΔPDE2Δ* mutant exhibited a 50 % increase in xylose consumption rate and a 70 % increase in ethanol production rate compared to the wild type [105]. Myers et al. demonstrated that the deletion of GTPase *IRA2* slowed the conversion of active RAS-GTP into inactive RAS-GDP, boosting anaerobic xylose utilization [100]. Deleting *BCY1*, encoding a negative regulatory subunit for PKA activity increased PKA activity, enabling xylose anaerobic fermentation, although cells could not grow anaerobically on xylose [91]. Subsequent verification revealed defective lipid homeostasis of the *BCY1Δ* mutant, leading to uncoupling between cell growth and metabolic pathways [106]. Transcriptomic analysis found that *BCY1* was associated with the iron-responsive transcription factor Aft1/2p and the inositol synthesis phospholipid-related transcription factor Ino4p [106,107].

In Myers et al.'s study, transcriptome and proteome of strains under aerobic and anaerobic conditions with different xylose metabolism characteristics revealed that 68 of 128 transcripts that were induced progressively stronger when shifted to anaerobic-xylose conditions were regulated by transcription factor *AZF1* which is responsible for different carbon source response. Elevated *AZF1* expression enhanced xylose anaerobic fermentation. Further analysis found reduced expression of *HAP4*, a respiratory regulation transcription factor, *MSN2/4*,stress response TFs, and the xylose transport inhibitor *MTH1* [91]. Moreover, the expression of the sterol and lipid synthesis-related transcription factor *MGA2* was upregulated. Examination confirmed that augmented *MGA2* expression stimulates xylose anaerobic fermentation [91,108]. Overexpression of *HAP4* or deletion of mitochondrial glycerol-3-P dehydrogenase *GUT2* also favors aerobic fermentation of xylose [109].

4. Transcription factor regulatory network alleviates antagonism between xylose utilization and strain robustness

The antagonistic relationship between xylose utilization ability and strain robustness is pervasive and challenging to eliminate [4,12,110]. Numerous studies have effectively alleviated antagonism by expressing functional genes and TFs [94,99,111]. Our conclusion is that the enhancement of xylose utilization under hydrolysate conditions depends on the antagonism between xylose utilization ability and strain robustness and the toxicity of hydrolysate.

In case where the hydrolysate is highly toxic, the growth and metabolism of strains with lower robustness are significantly inhibited when glucose is used as a carbon source. Therefore, the primary consideration should be to improve strain robustness to toxic hydrolysate. This is crucial as normal glucose utilization is a prerequisite for xylose utilization. Lam et al.'s investigation demonstrated the wild-type strain was insufficient to resist the toxicity of a simulated hydrolysate containing 100 mM acetic acid, furfural, and 5-HMF. Glucose remained at approximately 70 g L^{-1}, xylose around 25 g L^{-1}, and ethanol production at 22 g L^{-1}. However, by overproducing the evolved aldehyde dehydrogenase *GRE2* to increase stain robust, glucose and xylose were nearly completely utilized, yielding 66 g L^{-1} ethanol [112]. In our study, the low-robust engineered strain LF1 was fermented in a corn stalk hydrolysate (containing 4.3 g L^{-1} acetic acid, 5-HMF 1.0 g L^{-1}, furfural 0.35 g L^{-1} and 4.78 g L^{-1} phenolic compounds) with 50 g L^{-1} glucose and 25 g L^{-1} xylose, showing 50 % glucose consumption and almost no xylose consumption after 96 h. Although the xylose utilization ability of engineered strain 6M − 15 was weaker than LF1, 6M − 15 rapidly utilized glucose during the fermentation in this hydrolysate due to its high robustness, resulting in almost complete xylose consumption [113].

If the hydrolysate toxicity isn't sufficient to significantly hinder the strain's growth and metabolism when glucose is used as a carbon source, deliberately reducing the strain's robustness logically enhances its xylose utilization. It is possible that highly robust strains have excess resistance to inhibitors, and low-robust strains allocate excess resources to improve the xylose metabolism rate [109]. For instance, in corn bran hydrolysate containing 2.65 g L^{-1} acetic acid, 0.53 g L^{-1} furfural, 0.08 g L^{-1} 5-HMF and 2.01 g L^{-1} phenolic compounds, low-robust LF1 can metabolize glucose normally and exhibits significantly better xylose metabolic performance than high-robust 6M − 15 [114]. Additionally, our study showed that *HOG1Δ* mutants maximized xylose utilization compared to other *HOG1* mutants, with a corresponding significant

decrease in robustness. The PKA activity, stimulated by glucose in the cAMP-PKA pathway, facilitates anaerobic xylose fermentationbut does not enhance cell robustness, as it weakens cellular aerobic respiration and stress response resistance [91]. Moreover, the sufficient biomass accumulated during the glucose phase ensuresrapid xylose consumption. Results from many lignocellulose hydrolysate fermentations have shown that xylose enters a short period of rapid consumption due to activated PKA activity and accumulated biomass when glucose is nearly consumed, and xylose consumption rate becomes slower after glucose consumption [113–115]. To address this, strategies such as maintaining low glucose concentration in simultaneous saccharification fermentation, and fed-batch fermentation with low-concentration glucose addition or hydrolysate fermentation using recombinant yeast expressing β-glucosidase for converting cellobiose into glucose, have been used to promote xylose utilization, and increase ethanol production [116,117]. In our study, a recombinant strain expressing β-glucosidase improved xylose utilization by 50 % compared to the control strain in the cellobiose containing hydrolysate, attributed to the glucose-induced sugar signaling pathway [118]. Low glucose alleviates Mig1p′s repression of xylose metabolism, and the high activity of PKA caused by glucose activates the EMP pathway, driving xylose fermentation.

5. Transcription factor regulatory network mitigate post-glucose effect

Over the past few years, there have been notable advancements in improving the xylose metabolism rate of yeast in a xylose-only medium through metabolic pathway optimization and adaptive evolution. However, despite these efforts, the rate still lags significantly behind that of glucose. The post-glucose effect plays a crucial role in diminishing the xylose metabolism rate in hydrolysates.

5.1. Sugar sensitivity to glucose and xylose variance of S. cerevisiae

Upon introducing the xylose metabolic pathway in yeast, xylose undergoes transformation to xylose 5-P and integrates into the PPP pathway. However, some differences arise when yeast used glucose or xylose as carbon sources. In the presence of high concentrations of glucose or other fermented carbon sources such as maltose and sucrose, cells exhibit Crabtree-positive behavior [119]. In this state, non-fermenting carbon sources such as ethanol, glycerol, and acetic acid cannot be used. Conversely, under low fermented carbon sources, the cells become Crabtree-negative, and nonfermented carbon sources start being utilized. In yeast with xylose metabolizing capacity, glucose is preferentially used, followed by xylose, and finally nonfermented carbon sources. Salusjarvi et al. classify xylose as a semi-fermentative carbon source [120,121]. It is precisely because xylose has the properties of a nonfermented carbon source that it promotes the synthesis of terpenoids such as astaxanthin [122,123]. The metabolism of another pentose, arabinose, in lignocellulose, exihibits a similar metabolic order as xylose [124]. The underlying reason for the difference in metabolic order lies in the distinct signal of the sugar sensing signaling pathway. Sugar sensing signaling pathways Snf3p-Rgt2p, Snf1p-Mig1p and cAMP-PKA connect carbon source recognition with transcriptional regulation, forming interconnected signaling pathways [125]. While the response mechanism of the sugar signaling pathway to fermented and nonfermented carbon sources is well-documented, the response to xylose remains less studied. Brink and Osiro et al. show that high concentrations of xylose (50 g L^{-1}) can induce sugar signaling pathways as low concentrations of glucose (1 g L^{-1}) in recombinant yeast that metabolize xylose. The *IRA2ΔISU1Δ* mutant enhanced Snf3p-Rgt2p and cAMP-PKA signaling concurrently [86,126], highlighting the plasticity of the sugar signaling pathway under xylose carbon source [127].

Wei et al. conducted a study using haploid yeast BSGX01 and diploid yeast XH7 to analyze transcriptomic differences caused by the post-glucose effect. The study revealed diminished expression of genes involved in the EMP and PPP pathways during the GX phase. In contrast, the expression of genes involved in TCA, glyoxylic acid cycle, and electron transport chain was upregulated. Metabolic enzymes for the consumption of carbon sources such as fructose, galactose and sucrose were upregulated, while the expression of genes involved in ribosomal biogenesis and translation was downregulated. Importantly, the levels of sugar transport pathway Snf3p-Rgt2p in the sugar sensing pathway remained essentially unchanged, while the activities of non-fermentable carbon sources utilization pathway Snf1p-Mig1p and growth and fermentation pathway cAMP-PKA pathway were reduced [13]. The Snf1p-Mig1p pathway mainly regulates genes related to respiration, gluconeogenesis and selective carbon source metabolism. And the cAMP-PKA pathway emerged as the most important pathway influencing growth and fermentation [128]. Therefore, inhibition of the cAMP-PKA pathway is the fundamental factor of the post-glucose effect. When cells were stimulated by glucose, cAMP concentration increased rapidly, reaching a peak before decreasing to a level higher than the initial value [129,130]. As mentioned earlier, examples have been provided demonstrating the regulation of PKA activity to promote sugar metabolism, particularly in the work of Nguyen et al. [104]. Therefore, we infer that yeast cAMP also peaks when stimulated by xylose, but at a level below that observed when stimulated by glucose. This corresponds to a lower xylose metabolic rate compared to glucose. In a mixed sugar medium, yeast was stimulated by glucose, and cAMP concentration reached a peak before decreasing to a level higher than the initial value. Importantly, it did not rise again after complete utilization of glucose. This indicates that after glucose utilization, xylose no longer stimulates an increase of cAMP, aligning with the lower xylose metabolic performance in the GX stage compared to the X stage [13].

5.2. TFs responsible for regulation of post-glucose effect

TFs exhibiting co-variation at the X and GX stages of BSGX01 and XH7 were identified [13]. Subsequent validation revealed that deleting TFs related to carbon metabolism including *ARD1*, *ZNF1* and *NRG1* decreased xylose utilization, while deleting the transcription factor *THI2*, or overexpressing *NRM1* enhanced xylose utilization (Table 3). Specifically, the deletion of *THI2* in haploid yeast BSGX01 led to a 67.7 % increase in xylose utilization rate at GX stage, while causing a decrease at the X stage [13]. Therefore, *THI2* deletion was found to increase xylose metabolism primarily at the GX stage, rather than the X stage. Transcriptomic analysis of *THI2Δ* mutant found that the expression of Thi2p target genes including *THI4*, *THI5*, *THI4*, *THI6* and *THI20* was not changed at the GX stage, indicating that the improvement of xylose metabolism resulting from Thi2p deletion was unrelated to thiamine synthesis [131].

Upon verifying genes with significant upregulation in *THI2Δ* mutant, overexpression of cell wall integrity (CWI)-related genes containing *MID2*, *STT4* and *CDC42* was found to increase the xylose utilization rate by 45.9 %, 49.2 % and 13.1 %, respectively, at the GX stage. This analysis also revealed a noteworthy reduction in the proportion of dead cells in those mutants including the *THI2Δ* mutant at the GX stage. In addition, overexpression of stress response-related genes *ECM22*, *CSC1* and *BDH2* as well as RNA polymerase II/III synthesis-related genes *GPN3* and *TFC3*, and genes *BOP2* and *RGI2* with unknown function was

Table 3
Genes involved in alleviating post-glucose effect.

Genes	Gene function	Reference
THI2、	Cell survival rate	[13]
NRM1、*CIP1*、*YHP1*、*IXR1*	Cell cycle	[131]
ECM22、*CSC1*、*BDH2*、*IXR1*	Stress response	[131]
GPN3、*TFC3*	RNA polymerase synthesis	[131]
TEC1	Ribosome biogenesis	(data not shown)
TEC1	Metal ion absorption	(data not shown)

observed to increase xylose utilization by alleviating the post-glucose effect [131]. Deletion of *CIP1*, *IXR1*, *YDR246W-A* and *YGLO15C* which are down-regulated in the transcriptomic analysis resulted in an increased xylose utilization rate of 26.2 %, 36.1 %, 16.4 % and 14.8 %, respectively, at the GX stage. Notably, the deletion of *CIP1* accelerated the G1/S cell cycle transition [132,133], and overexpression of cell cycle-related genes *NRM1* and *YHP1* also demonstrated an alleviation in the post-glucose effect [13]. Deleteion of *IXR1*, encoded a protein associated with the cell cycle [134] upregulated the expression of stress-related genes [135], with this upregulation occurring exclusively during aerobic fermentation. The subsequent studies found that the post-glucose effect was also associated with transcription factor Tec1p mediated regulation of ribosome biosynthesis and iron absorption (data not shown). Nevertheless, more regulation mechanism and effective methods to alleviate post-glucose effect remain to be discovered.

6. Conclusions

S. cerevisiae stands out as the most promising strain for second-generation fuel ethanol production. However, the antagonism between high xylose utilization capacity and strain robustness, along with the post-glucose effect, poses challenges to efficient xylose utilization in lignocellulosic hydrolysates. We believe that *S. cerevisiae* with high robustness is indispensable, as investigations into the antagonistic mechanism reveal that improving the xylose utilization in hydrolysates often involves compromising robustness, a strategic trade-off to alleviate antagonism. Numerous TFs have been explored to overcome these bottlenecks, achieving some progress (Table 4). Although IF regulation has shown promising results, current strategies primarily target single molecular levels. Moving forward, identifying new TF targets, co-regulating multiple TFs, or dynamically regulating a single TF across different metabolic pathways may help balance xylose utilization and strain robustness while mitigating the post-glucose effect. For example, upregulating Hog1p enhances robustness, whereas downregulating it can improve xylose utilization. Thus, Hog1p expression could be required during the growth phase, followed by inducible inactivation during xylose utilization. Alternatively, studying the effect of gradient Hog1p expression under promoter control could help identify the optimal expression level that balances both xylose utilization and strain robustness. The inhibition of xylose metabolism by highly toxic hydrolysates, while it can be alleviated, remains resistant to complete elimination. To further improve fermentation efficiency, besides enhancement of xylose utilization and strain robustness, another focus should shift towards minimizing the toxicity of the hydrolysates.

CRediT authorship contribution statement

Dongming Sun: Writing – original draft, Methodology, Investigation. **Longhao Wu:** Methodology, Investigation. **Xiaocong Lu:** Methodology, Investigation. **Chenhao Li:** Methodology, Investigation. **Lili Xu:** Investigation. **Hongxing Li:** Investigation. **Deyun He:** Investigation. **Aiqun Yu:** Investigation. **Tao Yu:** Investigation. **Jianzhi Zhao:** Visualization, Supervision, Project administration, Funding acquisition, Conceptualization. **Hongting Tang:** Writing – review & editing, Project administration, Conceptualization. **Xiaoming Bao:** Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Data availability

Data will be made available on request.

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Table 4
Summary of TFs related to increase robustness, enhance xylose utilization and mitigate post-glucose effects.

Aim	Upregulation TFs	Downregulated TFs
Increase robustness	Hog1p, Msn2/4p, Yap1p, Haa1p, Stb5p, Ecm22p, Upc2p, War1p, Hsf1p, Sfp1p, Ume6p.	Yrr1p, Mig1p.
Enhance xylose utilization	Znf1p, Azf1p, Mga2p.	Hog1p, Ask10p, Tdh1p, Gcr2p.
Mitigate post-glucose effect	Nrm1p, Ecm22p, Yhp1p.	Thi2p, Ixr1p.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Wang D-N, Feng J, Yu C-X, Zhang X-K, Chen J, Wei L-J, et al. Integrated pathway engineering and transcriptome analysis for improved astaxanthin biosynthesis in Yarrowia lipolytica. Synthetic and Systems Biotechnology 2022;7:1133–41.
[2] Yu J, Lv D, Zhang L. De novo biosynthesis of a vinblastine precursor in Pichia pastoris. Synthetic and Systems Biotechnology 2023;8:300–1.
[3] Fiamenghi MB, Bueno JGR, Camargo AP, Borelli G, Carazzolle MF, Pereira GAG, et al. Machine learning and comparative genomics approaches for the discovery of xylose transporters in yeast. Biotechnol Biofuels Bioprod 2022;15:57.
[4] Demeke MM, Dietz H, Li Y, Foulquié-Moreno MR, Mutturi S, Deprez S, et al. Development of a D-xylose fermenting and inhibitor tolerant industrial *Saccharomyces cerevisiae* strain with high performance in lignocellulose hydrolysates using metabolic and evolutionary engineering. Biotechnol Biofuels 2013;6:89.
[5] Chen S, Xu Z, Ding B, Zhang Y, Liu S, Cai C, et al. Big data mining, rational modification, and ancestral sequence reconstruction inferred multiple xylose isomerases for biorefinery. Sci Adv 2023;9:eadd8835.
[6] Li H, Shen Y, Wu M, Hou J, Jiao C, Li Z, et al. Engineering a wild-type diploid *Saccharomyces cerevisiae* strain for second-generation bioethanol production. Bioresour Bioprocess. 2016;3:51.
[7] Ding B, Xu Z, Chen S, Li M, Cai C, Zhang Y, et al. Quantitative understanding of the impact of stress factors on xylose fermentation at different high solid biomass loads. Ind Crop Prod 2023;203:117134.
[8] Zhang Y, Xu Z, Lu M, Ding B, Chen S, Wen Z, et al. Rapid evolution and mechanism elucidation for efficient cellobiose-utilizing *Saccharomyces cerevisiae* through synthetic chromosome rearrangement and modification by LoxPsym-mediated evolution. Bioresour Technol 2022;356:127268.
[9] Cámara E, Olsson L, Zrimec J, Zelezniak A, Geijer C, Nygård Y. Data mining of *Saccharomyces cerevisiae* mutants engineered for increased tolerance towards inhibitors in lignocellulosic hydrolysates. Biotechnol Adv 2022;57:107947.
[10] Guo H, Zhao Y, Chang J-S, Lee D-J. Inhibitor formation and detoxification during lignocellulose biorefinery: a review. Bioresour Technol 2022;361:127666.
[11] Koppram R, Albers E, Olsson L. Evolutionary engineering strategies to enhance tolerance of xylose utilizing recombinant yeast to inhibitors derived from spruce biomass. Biotechnol Biofuels 2012;5:32.
[12] Wei F, Li M, Wang M, Li H, Li Z, Qin W, et al. A C6/C5 co-fermenting *Saccharomyces cerevisiae* strain with the alleviation of antagonism between xylose utilization and robustness. GCB Bioenergy 2021;13:83–97.
[13] Wei S, Liu Y, Wu M, Ma T, Bai X, Hou J, et al. Disruption of the transcription factors Thi2p and Nrm1p alleviates the post-glucose effect on xylose utilization in *Saccharomyces cerevisiae*. Biotechnol Biofuels 2018;11:112.
[14] Shan Wei. Study on the xylose metabolic efficiency decisive factors relevant to the post-glucose-effect in *Saccharomyces cerevisiae*. Shandong University; 2019.
[15] Caspeta L, Castillo T, Nielsen J. Modifying yeast tolerance to inhibitory conditions of ethanol production processes. Front Bioeng Biotechnol 2015;3184.
[16] Lam FH, Ghaderi A, Fink GR, Stephanopoulos G. Engineering alcohol tolerance in yeast. Science. 2014;346:71–5.
[17] Geng P, Zhang L, Shi GY. Omics analysis of acetic acid tolerance in *Saccharomyces cerevisiae*. World J Microbiol Biotechnol 2017;33:94.
[18] Procópio DP, Lee JW, Shin J, Tramontina R, Ávila PF, Brenelli LB, et al. Metabolic engineering of *Saccharomyces cerevisiae* for second-generation ethanol production from xylo-oligosaccharides and acetate. Sci Rep 2023;13:19182.
[19] Qi L, Zhu Y-X, Wang Y-K, Tang X-X, Li K-J, He M, et al. Nonlethal furfural exposure causes genomic alterations and adaptability evolution in *Saccharomyces cerevisiae*Cuomo CA, editor. Microbiol Spectr 2023;11:e01216–23.

[20] Jayakody LN, Jin Y-S. In-depth understanding of molecular mechanisms of aldehyde toxicity to engineer robust *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 2021;105:2675–92.
[21] López PC, Peng C, Arneborg N, Junicke H, Gernaey KV. Analysis of the response of the cell membrane of *Saccharomyces cerevisiae* during the detoxification of common lignocellulosic inhibitors. Sci Rep 2021;11:6853.
[22] Luo H, Liu Z, Xie F, Bilal M, Peng F. Lignocellulosic biomass to biobutanol: toxic effects and response mechanism of the combined stress of lignin-derived phenolic acids and phenolic aldehydes to Clostridium acetobutylicum. Ind Crop Prod 2021; 170:113722.
[23] Wolf IR, Marques LF, De Almeida LF, Lázari LC, De Moraes LN, Cardoso LH, et al. Integrative analysis of the ethanol tolerance of *Saccharomyces cerevisiae*. IJMS 2023;24:5646.
[24] Liu ZL, Slininger PJ, Dien BS, Berhow MA, Kurtzman CP, Gorsich SW. Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2,5-bis-hydroxymethylfuran. J IND MICROBIOL BIOTECHNOL 2004;31:345–52.
[25] Gu H, Zhu Y, Peng Y, Liang X, Liu X, Shao L, et al. Physiological mechanism of improved tolerance of *Saccharomyces cerevisiae* to lignin-derived phenolic acids in lignocellulosic ethanol fermentation by short-term adaptation. Biotechnol Biofuels 2019;12:268.
[26] Chen Y, Sheng J, Jiang T, Stevens J, Feng X, Wei N. Transcriptional profiling reveals molecular basis and novel genetic targets for improved resistance to multiple fermentation inhibitors in *Saccharomyces cerevisiae*. Biotechnol Biofuels 2016;9:9.
[27] Takayama T, Yamamoto K, Saito H, Tatebayashi K. Interaction between the transmembrane domains of Sho1 and Opy2 enhances the signaling efficiency of the Hog1 MAP kinase cascade in *Saccharomyces cerevisiae*Sugiura R, editor. PLoS One 2019;14:e0211380.
[28] Du G, Zhang X, Gao Y, Sun C, Wang L, Zhao W, et al. Heat shock transcriptional factor HSF1 is activated by phosphorylation in response to ginger oleoresin stress in *S. cerevisiae*. LWT 2023;184:115116.
[29] Wu G, Xu Z, Jönsson LJ. Profiling of *Saccharomyces cerevisiae* transcription factors for engineering the resistance of yeast to lignocellulose-derived inhibitors in biomass conversion. Microb Cell Factories 2017;16:199.
[30] Cunha JT, Costa CE, Ferraz L, Romaní A, Johansson B, Sá-Correia I, et al. HAA1 and PRS3 overexpression boosts yeast tolerance towards acetic acid improving xylose or glucose consumption: unravelling the underlying mechanisms. Appl Microbiol Biotechnol 2018;102:4589–600.
[31] Bergman A, Vitay D, Hellgren J, Chen Y, Nielsen J, Siewers V. Effects of overexpression of *STB5* in *Saccharomyces cerevisiae* on fatty acid biosynthesis, physiology and transcriptome. FEMS Yeast Res 2019;19:foz027.
[32] Jordá T, Barba-Aliaga M, Rozès N, Alepuz P, Martínez-Pastor MT, Puig S. Transcriptional regulation of ergosterol biosynthesis genes in response to iron deficiency. Environ Microbiol 2022;24:5248–60.
[33] Kren A, Mamnun YM, Bauer BE, Schüller C, Wolfger H, Hatzixanthis K, et al. War1p, a novel transcription factor controlling weak acid stress response in yeast. Mol Cell Biol 2003;23:1775–85.
[34] Lettow J, Kliewe F, Aref R, Schüller H-J. Functional characterization and comparative analysis of gene repression-mediating domains interacting with yeast pleiotropic corepressors Sin3, Cyc8 and Tup1. Curr Genet 2023;69:127–39.
[35] Guo Z, Li M, Guo Z, Zhu R, Xin Y, Gu Z, et al. Trehalose metabolism targeting as a novel strategy to modulate acid tolerance of yeasts and its application in food industry. Food Microbiol 2023;114:104300.
[36] Wang X, Liang Z, Hou J, Shen Y, Bao X. The absence of the transcription factor Yrr1p, identified from comparative genome profiling, increased vanillin tolerance due to enhancements of ABC transporters expressing, rRNA processing and ribosome biogenesis in *Saccharomyces cerevisiae*. Front Microbiol 2017;8367.
[37] Marion RM, Regev A, Segal E, Barash Y, Koller D, Friedman N, et al. Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. Proc Natl Acad Sci USA 2004;101:14315–22.
[38] Cheng Y, Zhu H, Du Z, Guo X, Zhou C, Wang Z, et al. Eukaryotic translation factor eIF5A contributes to acetic acid tolerance in *Saccharomyces cerevisiae* via transcriptional factor Ume6p. Biotechnol Biofuels 2021;14:38.
[39] Westfall PJ, Thorner J. Analysis of mitogen-activated protein kinase signaling specificity in response to hyperosmotic stress: use of an analog-sensitive *HOG1* allele. Eukaryot Cell 2006;5:1215–28.
[40] Weng F, Wang Y. Regulation of mitogen-activated protein kinase Hog1 phosphorylation induced by DNA damage agent in *Saccharomyces cerevisiae*. Faseb J 2022;36. fasebj.2022.36.S1.0R403.
[41] Parmar JH, Bhartiya S, Venkatesh KV. A model-based study delineating the roles of the two signaling branches of *Saccharomyces cerevisiae* , Sho1 and Sln1, during adaptation to osmotic stress. Phys Biol 2009;6:036019.
[42] Sone M, Navanopparatsakul K, Takahashi S, Furusawa C, Hirasawa T. Loss of function of Hog1 improves glycerol assimilation in *Saccharomyces cerevisiae*. World J Microbiol Biotechnol 2023;39:255.
[43] Bai C, Tesker M, Melamed-Kadosh D, Engelberg D, Admon A. Hog1-induced transcription of *RTC3* and *HSP12* is robust and occurs in cells lacking Msn2, Msn4, Hot1 and Sko1. In: Mata J, editor. PLoS ONE, vol. 15; 2020, e0237540.
[44] Holyavkin C, Turanlı-Yıldız B, Yılmaz Ü, Alkım C, Arslan M, Topaloğlu A, et al. Genomic, transcriptomic, and metabolic characterization of 2-Phenylethanol-resistant *Saccharomyces cerevisiae* obtained by evolutionary engineering. Front Microbiol 2023;14:1148065.
[45] Mosbacher M, Lee SS, Yaakov G, Nadal-Ribelles M, De Nadal E, Van Drogen F, et al. Positive feedback induces switch between distributive and processive phosphorylation of Hog1. Nat Commun 2023;14:2477.
[46] Chen H-Q, Xing Q, Cheng C, Zhang M-M, Liu C-G, Champreda V, et al. Identification of Kic1p and Cdc42p as novel targets to engineer yeast acetic acid stress tolerance. Front Bioeng Biotechnol 2022;10:837813.
[47] Nasution O, Lee YM, Kim E, Lee Y, Kim W, Choi W. Overexpression of *OLE1* enhances stress tolerance and constitutively activates the MAPK HOG pathway in *Saccharomyces cerevisiae*. Biotechnol Bioeng 2017;114:620–31.
[48] Westfall PJ, Ballon DR, Thorner J. When the stress of your environment makes you go HOG wild. Science 2004;306:1511–2.
[49] Yang P, Jiang S, Jiang S, Lu S, Zheng Z, Chen J, et al. CRISPR-Cas9 approach constructed engineered *Saccharomyces cerevisiae* with the deletion of *GPD2, FPS1*, and *ADH2* to enhance the production of ethanol. JoF 2022;8:703.
[50] Nishimura A, Tanahashi R, Nakazawa H, Oi T, Mima M, Takagi H. PKA-Msn2/4-Shy1 cascade controls inhibition of proline utilization under wine fermentation models. J Biosci Bioeng 2023:S1389172323003237.
[51] Yang G, Liu G-L, Wang S-J, Chi Z-M, Chi Z. Pullulan biosynthesis in yeast-like fungal cells is regulated by the transcriptional activator Msn2 and cAMP-PKA signaling pathway. Int J Biol Macromol 2020;157:591–603.
[52] Jacquet M, Renault G, Lallet S, De Mey J, Goldbeter A. Oscillatory nucleocytoplasmic shuttling of the general stress response transcriptional activators Msn2 and Msn4 in *Saccharomyces cerevisiae*. J Cell Biol 2003;161: 497–505.
[53] Berry DB, Gasch AP. Stress-activated genomic expression changes serve a preparative role for impending stress in yeastWeissman JS, editor. MBoC 2008; 19:4580–7.
[54] Van Dijk M, Rugbjerg P, Nygård Y, Olsson L. RNA sequencing reveals metabolic and regulatory changes leading to more robust fermentation performance during short-term adaptation of *Saccharomyces cerevisiae* to lignocellulosic inhibitors. Biotechnol Biofuels 2021;14:201.
[55] Varize CS, Bücker A, Lopes LD, Christofoleti-Furlan RM, Raposo MS, Basso LC, et al. Increasing ethanol tolerance and ethanol production in an industrial fuel ethanol *Saccharomyces cerevisiae* strain. Fermentation 2022;8:470.
[56] Sadeh A, Baran D, Volokh M, Aharoni A. Conserved motifs in the msn2-activating domain are important for msn2-mediated yeast stress response. J Cell Sci 2012: 096446.
[57] Duy DL, Kim N. Yeast transcription factor Msn2 binds to G4 DNA. Nucleic Acids Res 2023;51:9643–57.
[58] Kyriakou M, Christodoulou M, Ioannou A, Fotopoulos V, Koutinas M. Improvement of stress multi-tolerance and bioethanol production by *Saccharomyces cerevisiae* immobilised on biochar: monitoring transcription from defence-related genes. Biochem Eng J 2023;195:108914.
[59] Monteiro G, Netto LES. Glucose repression of *PRX1* expression is mediated by Tor1p and Ras2p through inhibition of Msn2/4p in *Saccharomyces cerevisiae*. FEMS (Fed Eur Microbiol Soc) Microbiol Lett 2004;241:221–8.
[60] Wong C-M, Ching Y-P, Zhou Y, Kung H-F, Jin D-Y. Transcriptional regulation of yeast peroxiredoxin gene TSA2 through Hap1p, Rox1p, and Hap2/3/5p. Free Radic Biol Med 2003;34:585–97.
[61] Qin L, Dong S, Yu J, Ning X, Xu K, Zhang S-J, et al. Stress-driven dynamic regulation of multiple tolerance genes improves robustness and productive capacity of *Saccharomyces cerevisiae* in industrial lignocellulose fermentation. Metab Eng 2020;61:160–70.
[62] Mat Nanyan NSB, Watanabe D, Sugimoto Y, Takagi H. Involvement of the stress-responsive transcription factor gene *MSN2* in the control of amino acid uptake in *Saccharomyces cerevisiae*. FEMS Yeast Res 2019;19:foz052.
[63] Sundström L, Larsson S, Jönsson LJ. Identification of *Saccharomyces cerevisiae* genes involved in the resistance to phenolic fermentation inhibitors. Appl Biochem Biotechnol 2010;161:106–15.
[64] Wang H, Li Q, Kuang X, Xiao D, Han X, Hu X, et al. Functions of aldehyde reductases from *Saccharomyces cerevisiae* in detoxification of aldehyde inhibitors and their biotechnological applications. Appl Microbiol Biotechnol 2018;102: 10439–56.
[65] Zhang M-M, Xiong L, Tang Y-J, Mehmood MA, Zhao ZK, Bai F-W, et al. Enhanced acetic acid stress tolerance and ethanol production in *Saccharomyces cerevisiae* by modulating expression of the de novo purine biosynthesis genes. Biotechnol Biofuels 2019;12:116.
[66] Zheng D-Q, Jin X-N, Zhang K, Fang Y-H, Wu X-C. Novel strategy to improve vanillin tolerance and ethanol fermentation performances of Saccharomycere cerevisiae strains. Bioresour Technol 2017;231:53–8.
[67] Divate NR, Chen G-H, Wang P-M, Ou B-R, Chung Y-C. Engineering *Saccharomyces cerevisiae* for improvement in ethanol tolerance by accumulation of trehalose. Bioengineered 2016;7:445–58.
[68] Kong M, Li X, Li T, Zhao X, Jin M, Zhou X, et al. Overexpressing *CCW12* in *Saccharomyces cerevisiae* enables highly efficient ethanol production from lignocellulose hydrolysates. Bioresour Technol 2021;337:125487.
[69] Huang Z, Yu Y, Fang Z, Deng Y, Shen Y, Shi P. OLE1 reduces cadmium-induced oxidative damage in *Saccharomyces cerevisiae*. FEMS Microbiol Lett 2018;365:18.
[70] Zhang Y, Pang J, Liu S, Nie K, Deng L, Wang F, et al. Harnessing transcription factor Mga2 and fatty acid elongases to overproduce palmitoleic acid in *Saccharomyces cerevisiae*. Biochem Eng J 2022;181:108402.
[71] Mira NP, Becker JD, Sá-Correia I. Genomic expression Program involving the haa1p-regulon in *Saccharomyces cerevisiae* response to acetic acid. OMICS A J Integr Biol 2010;14:587–601.
[72] Antunes M, Palma M, Sá-Correia I. Transcriptional profiling of Zygosaccharomyces bailii early response to acetic acid or copper stress mediated by ZbHaa1. Sci Rep 2018;8:14122.

[73] Tanaka K, Ishii Y, Ogawa J, Shima J. Enhancement of acetic acid tolerance in *Saccharomyces cerevisiae* by overexpression of the *HAA1* gene, encoding a transcriptional activator. Appl Environ Microbiol 2012;78:8161–3.

[74] Swinnen S, Henriques SF, Shrestha R, Ho P-W, Sá-Correia I, Nevoigt E. Improvement of yeast tolerance to acetic acid through Haa1 transcription factor engineering: towards the underlying mechanisms. Microb Cell Factories 2017;16:7.

[75] Martínez-Muñoz GA, Kane P. Vacuolar and plasma membrane proton pumps collaborate to achieve cytosolic pH homeostasis in yeast. J Biol Chem 2017;292:7743.

[76] Balderas-Hernández VE, Correia K, Mahadevan R. Inactivation of the transcription factor *mig1* (*YGL035C*) in *Saccharomyces cerevisiae* improves tolerance towards monocarboxylic weak acids: acetic, formic and levulinic acid. J Ind Microbiol Biotechnol 2018;45:735–51.

[77] Yin Y, Zhang H, Zhang Y, Hu C, Sun X, Liu W, et al. Fungal Zn(II) $_2$ cys $_6$ transcription factor ADS-1 regulates drug efflux and ergosterol metabolism under antifungal azole stress. Antimicrob Agents Chemother 2021;65.

[78] Kodo N, Matsuda T, Doi S, Munakata H. Salicylic acid resistance is conferred by a novel YRR1 mutation in *Saccharomyces cerevisiae*. Biochem Biophys Res Commun 2013;434:42–7.

[79] Shrivastava M, Kouyoumdjian GS, Kirbizakis E, Ruiz D, Henry M, Vincent AT, et al. The Adr1 transcription factor directs regulation of the ergosterol pathway and azole resistance in *Candida albicans*Goldman GH, editor. mBio 2023;14.

[80] Cao W, Zhao W, Yang B, Wang X, Shen Y, Wei T, et al. Proteomic analysis revealed the roles of YRR1 deletion in enhancing the vanillin resistance of *Saccharomyces cerevisiae*. Microb Cell Factories 2021;20:142.

[81] Zhao W, Wang X, Yang B, Wang Y, Li Z, Bao X. Unravel the regulatory mechanism of Yrr1p phosphorylation in response to vanillin stress in *Saccharomyces cerevisiae*. Microb Cell Factories 2023;22:48.

[82] Kocaefe-Özşen N, Yilmaz B, Alkım C, Arslan M, Topaloğlu A, Kısakesen HL, et al. Physiological and Molecular Characterization of an Oxidative Stress-Resistant *Saccharomyces cerevisiae* Strain Obtained by Evolutionary Engineering. Front Microbiol 2022;13:822864.

[83] Iwaki A, Ohnuki S, Suga Y, Izawa S, Ohya Y. Vanillin inhibits translation and induces messenger ribonucleoprotein (mRNP) granule formation in *Saccharomyces cerevisiae*: application and validation of high-content, image-based profilingGranneman S, editor. PLoS One 2013;8:e61748.

[84] Song Q-X, Liu N-N, Liu Z-X, Zhang Y-Z, Rety S, Hou X-M, et al. Nonstructural N- and C-tails of Dbp2 confer the protein full helicase activities. J Biol Chem 2023;299:104592.

[85] Nijland JG, Zhang X, Driessen AJM. d-xylose accelerated death of pentose metabolizing *Saccharomyces cerevisiae*. Biotechnol Biofuels 2023;16:67.

[86] Osiro KO, Borgström C, Brink DP, Fjölnisdóttir BL, Gorwa-Grauslund MF. Exploring the xylose paradox in *Saccharomyces cerevisiae* through in vivo sugar signalomics of targeted deletants. Microb Cell Factories 2019;18:88.

[87] Hou J, Jiao C, Peng B, Shen Y, Bao X. Mutation of a regulator Ask10p improves xylose isomerase activity through up-regulation of molecular chaperones in *Saccharomyces cerevisiae*. Metab Eng 2016;38:241–50.

[88] Randez-Gil F, Sánchez-Adriá IE, Estruch F, Prieto JA. The formation of hybrid complexes between isoenzymes of glyceraldehyde-3-phosphate dehydrogenase regulates its aggregation state, the glycolytic activity and sphingolipid status in *Saccharomyces cerevisiae*. Microb Biotechnol 2020;13:562–71.

[89] Shin M, Park H, Kim S, Oh EJ, Jeong D, Florencia C, et al. Transcriptomic changes induced by deletion of transcriptional regulator *GCR2* on pentose sugar metabolism in *Saccharomyces cerevisiae*. Front Bioeng Biotechnol 2021;9:654177.

[90] Songdech P, Intasit R, Yingchutrakul Y, Butkinaree C, Ratanakhanokchai K, Soontorngun N. Activation of cryptic xylose metabolism by a transcriptional activator Znf1 boosts up xylitol production in the engineered *Saccharomyces cerevisiae* lacking xylose suppressor BUD21 gene. Microb Cell Factories 2022;21:32.

[91] Myers KS, Riley NM, MacGilvray ME, Sato TK, McGee M, Heilberger J, et al. Rewired cellular signaling coordinates sugar and hypoxic responses for anaerobic xylose fermentation in yeastDudley AM, editor. PLoS Genet 2019;15:e1008037.

[92] Dos Santos LV, Carazzolle MF, Nagamatsu ST, Sampaio NMV, Almeida LD, Pirolla RAS, et al. Unraveling the genetic basis of xylose consumption in engineered *Saccharomyces cerevisiae* strains. Sci Rep 2016;6:38676.

[93] Murakami Y, Tatebayashi K, Saito H. Two adjacent docking sites in the yeast Hog1 mitogen-activated protein (MAP) kinase differentially interact with the Pbs2 MAP kinase kinase and the Ptp2 protein tyrosine phosphatase. Mol Cell Biol 2008;28:2481–94.

[94] Li X, Wang Y, Li G, Liu Q, Pereira R, Chen Y, et al. Metabolic network remodelling enhances yeast's fitness on xylose using aerobic glycolysis. Nat Catal 2021;4:783–96.

[95] Wei N, Xu H, Kim SR, Jin Y-S. Deletion of *FPS1* , encoding aquaglyceroporin Fps1p, improves xylose fermentation by engineered *Saccharomyces cerevisiae*. Appl Environ Microbiol 2013;79:3193–201.

[96] Xiong M, Chen G, Barford J. Genetic engineering of yeasts to improve ethanol production from xylose. J Taiwan Inst Chem Eng 2014;45:32–9.

[97] Usher J, Balderas-Hernandez V, Quon P, Gold ND, Martin VJJ, Mahadevan R, et al. Chemical and synthetic genetic array analysis identifies genes that suppress xylose utilization and fermentation in *Saccharomyces cerevisiae*. G3 Genes Genomes Genetics. 2011;1:247–58.

[98] Songdech P, Ruchala J, Semkiv MV, Jensen LT, Sibirny A, Ratanakhanokchai K, et al. Overexpression of transcription factor *ZNF1* of glycolysis improves bioethanol productivity under high glucose concentration and enhances acetic acid tolerance of *Saccharomyces cerevisiae*. Biotechnol J 2020;15:1900492.

[99] Palermo GCDL, Coutouné N, Bueno JGR, Maciel LF, Dos Santos LV. Exploring metal ion metabolisms to improve xylose fermentation in *Saccharomyces cerevisiae*. Microb Biotechnol 2021;14:2101–15.

[100] Sato TK, Tremaine M, Parreiras LS, Hebert AS, Myers KS, Higbee AJ, et al. Directed evolution reveals unexpected epistatic interactions that alter metabolic regulation and enable anaerobic xylose use by *Saccharomyces cerevisiae*Caudy A, editor. PLoS Genet 2016;12:e1006372.

[101] Soto IC, Fontanesi F, Myers RS, Hamel P, Barrientos A. A heme-sensing mechanism in the translational regulation of mitochondrial cytochrome c oxidase biogenesis. Cell Metabol 2012;16:801–13.

[102] Li L, Ward DM. Iron toxicity in yeast: transcriptional regulation of the vacuolar iron importer Ccc1. Curr Genet 2018;64:413–6.

[103] Dihazi H, Kessler R, Eschrich K. Glucose-induced stimulation of the ras-cAMP pathway in yeast leads to multiple phosphorylations and activation of 6-Phosphofructo-2-kinase. Biochemistry 2003;42:6275–82.

[104] Nguyen V, Xue P, Li Y, Zhao H, Lu T. Controlling circuitry underlies the growth optimization of *Saccharomyces cerevisiae*. Metab Eng 2023;80:173–83.

[105] Wu M, Li H, Wei S, Wu H, Wu X, Bao X, et al. Simulating extracellular glucose signals enhances xylose metabolism in recombinant *Saccharomyces cerevisiae*. Microorganisms 2020;8:100.

[106] Wagner ER, Nightingale NM, Jen A, Overmyer KA, McGee M, Coon JJ, et al. PKA regulatory subunit Bcy1 couples growth, lipid metabolism, and fermentation during anaerobic xylose growth in *Saccharomyces cerevisiae*Fay JC, editor. PLoS Genet 2023;19:e1010593.

[107] Martins TS, Costa V, Pereira C. Signaling pathways governing iron homeostasis in budding yeast. Mol Microbiol 2018;109:422–32.

[108] Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, et al. The genetic landscape of a cell. Science 2010;327:425–31.

[109] Li X, Wang Y, Li G, Liu Q, Pereira R, Chen Y, et al. Metabolic network remodelling enhances yeast's fitness on xylose using aerobic glycolysis. Nat Catal 2021;4:783–96.

[110] Barros KO, Mader M, Krause DJ, Pangilinan J, Andreopoulos B, Lipzen A, et al. Oxygenation influences xylose fermentation and gene expression in the yeast genera Spathaspora and Scheffersomyces. Biotechnol Biofuels 2024;17:20.

[111] Cheng C, Wang W, Sun M, Tang R, Bai L, Alper HS, et al. Deletion of *NGG1* in a recombinant *Saccharomyces cerevisiae* improved xylose utilization and affected transcription of genes related to amino acid metabolism. Front Microbiol 2022;13:960114.

[112] Lam FH, Turanlı-Yıldız B, Liu D, Resch MG, Fink GR, Stephanopoulos G. Engineered yeast tolerance enables efficient production from toxified lignocellulosic feedstocks. Sci Adv 2021;7:eabf7613.

[113] Wei F, Li M, Wang M, Li H, Li Z, Qin W, et al. A C6/C5 co-fermenting *Saccharomyces cerevisiae* strain with the alleviation of antagonism between xylose utilization and robustness. GCB Bioenergy 2021;13:83–97.

[114] Li M, Xu F, Zhao Y, Sun D, Liu J, Yin X, et al. High-efficient production of cellulosic ethanol from corn fiber based on the suitable C5/C6 Co-fermentation *Saccharomyces cerevisiae* strain. Fermentation 2023;9:743.

[115] Chen S, Xu Z, Ding B, Zhang Y, Liu S, Cai C, et al. Big data mining, rational modification, and ancestral sequence reconstruction inferred multiple xylose isomerases for biorefinery. Sci Adv 2023;9:eadd8835.

[116] Bertilsson M, Olofsson K, Lidén G. Prefermentation improves xylose utilization in simultaneous saccharification and co-fermentation of pretreated spruce. Biotechnol Biofuels 2009;2:8.

[117] Lopez PC, Abeykoon Udugama I, Thomsen ST, Bayer C, Junicke H, Gernaey KV. Promoting the co-utilisation of glucose and xylose in lignocellulosic ethanol fermentations using a data-driven feed-back controller. Biotechnol Biofuels 2020;13:190.

[118] Zhao J, Zhao Y, Wu L, Yan N, Yang S, Xu L, et al. Development of a robust *Saccharomyces cerevisiae* strain for efficient Co-fermentation of mixed sugars and enhanced inhibitor tolerance through protoplast fusion. Microorganisms 2024;12:1526.

[119] Lee S-B, Tremaine M, Place M, Liu L, Pier A, Krause DJ, et al. Crabtree/Warburg-like aerobic xylose fermentation by engineered *Saccharomyces cerevisiae*. Metab Eng 2021;68:119–30.

[120] Salusjärvi L, Kankainen M, Soliymani R, Pitkänen J-P, Penttilä M, Ruohonen L. Regulation of xylose metabolism in recombinant *Saccharomyces cerevisiae*. Microb Cell Factories 2008;7:18.

[121] Salusjärvi L, Pitkänen J-P, Aristidou A, Ruohonen L, Penttilä M. Transcription analysis of recombinant *Saccharomyces cerevisiae* reveals novel responses to xylose. ABAB 2006;128:237–74.

[122] Basiony M, Ouyang L, Wang D, Yu J, Zhou L, Zhu M, et al. Optimization of microbial cell factories for astaxanthin production: biosynthesis and regulations, engineering strategies and fermentation optimization strategies. Synthetic and Systems Biotechnology 2022;7:689–704.

[123] Muñoz-Fernández G, Martínez-Buey R, Revuelta JL, Jiménez A. Metabolic engineering of Ashbya gossypii for limonene production from xylose. Biotechnol Biofuels 2022;15:79.

[124] Verhoeven MD, De Valk SC, Daran J-MG, Van Maris AJA, Pronk JT. Fermentation of glucose-xylose-arabinose mixtures by a synthetic consortium of single-sugar-fermenting *Saccharomyces cerevisiae* strains. FEMS Yeast Res 2018;18:8.

[125] Gancedo JM, Flores C-L, Gancedo C. The repressor Rgt1 and the cAMP-dependent protein kinases control the expression of the *SUC2* gene in *Saccharomyces cerevisiae*. Biochim Biophys Acta Gen Subj 2015;1850:1362–7.

[126] Brink DP, Borgström C, Tueros FG, Gorwa-Grauslund MF. Real-time monitoring of the sugar sensing in *Saccharomyces cerevisiae* indicates endogenous mechanisms for xylose signaling. Microb Cell Factories 2016;15:183.

[127] Shen Y, Hou J, Bao X. Enhanced xylose fermentation capacity related to an altered glucose sensing and repression network in a recombinant *Saccharomyces cerevisiae*. Bioengineered 2013;4:435–7.
[128] Busti S, Coccetti P, Alberghina L, Vanoni M. Glucose signaling-mediated coordination of cell growth and cell cycle in *Saccharomyces cerevisiae*. Sensors 2010;10:6195–240.
[129] Gonzales K, Kayıkçı Ö, Schaeffer DG, Magwene PM. Modeling mutant phenotypes and oscillatory dynamics in the *Saccharomyces cerevisiae* cAMP-PKA pathway. BMC Syst Biol 2013;7:40.
[130] Ma P, Wera S, Van Dijck P, Thevelein JM. The *PDE1* -encoded low-affinity phosphodiesterase in the yeast *Saccharomyces cerevisiae* has a specific function in controlling agonist-induced cAMP signalingGuidotti G, editor. MBoC 1999;10:91–104.
[131] Wei S, Bai P, Liu Y, Yang M, Ma J, Hou J, et al. A Thi2p regulatory network controls the post-glucose effect of xylose utilization in *Saccharomyces cerevisiae*. Front Microbiol 2019;10:1649.
[132] Chang Y-L, Tseng S-F, Huang Y-C, Shen Z-J, Hsu P-H, Hsieh M-H, et al. Yeast Cip1 is activated by environmental stress to inhibit Cdk1–G1 cyclins via Mcm1 and Msn2/4. Nat Commun 2017;8:56.
[133] De Blasio C, Verma N, Moretti M, Cialfi S, Zonfrilli A, Franchitto M, et al. Functional cooperation between ASK1 and p21Waf1/Cip1 in the balance of cell-cycle arrest, cell death and tumorigenesis of stressed keratinocytes. Cell Death Dis 2021;7:75.
[134] Sato M, Irie K, Suda Y, Mizuno T, Irie K. The RNA-binding protein Puf5 and the HMGB protein Ixr1 contribute to cell cycle progression through the regulation of cell cycle-specific expression of CLB1 in *Saccharomyces cerevisiae*AP Mitchell, editor. PLoS Genet 2022;18:e1010340.
[135] Vizoso-Vázquez Á, Lamas-Maceiras M, Becerra M, González-Siso MI, Rodríguez-Belmonte E, Cerdán ME. Ixr1p and the control of the *Saccharomyces cerevisiae* hypoxic response. Appl Microbiol Biotechnol 2012;94:173–84.