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Comparison of stress tolerance mechanisms between *Saccharomyces cerevisiae* and the multistress-tolerant *Pichia kudriavzevii*

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Abstract

Yeasts play a vital role in both research and industrial biomanufacturing. *Saccharomyces cerevisiae* has been extensively utilized as a model system. However, its application is often constrained by limited tolerance to the diverse stress conditions encountered in bioprocesses. These challenges have driven increasing interest in nonconventional, multistress-tolerant yeasts as alternative biomanufacturing hosts. This review highlights *Pichia kudriavzevii* as a promising nonconventional yeast for industrial applications. Unlike *S. cerevisiae*, *P. kudriavzevii* exhibits exceptional tolerance to high temperatures, elevated concentrations of furanic and phenolic inhibitors, osmotic stress, salinity, and extreme pH. These traits make it an attractive candidate for industrial processes without requiring extensive genetic modifications to enhance stress resistance. As a result, *P. kudriavzevii* has emerged as a flagship species for advancing bioeconomy. Despite its industrial potential, the molecular mechanisms underlying *P. kudriavzevii*'s superior stress tolerance remain poorly understood. This review compiles current knowledge on *P. kudriavzevii* and compares its stress tolerance mechanisms with those of *S. cerevisiae*, providing insights into its innate resilience. By expanding our understanding of nonconventional yeasts, this review aims to facilitate their broader adoption as robust microbial platforms for industrial biomanufacturing.

Keywords: *Saccharomyces cerevisiae*; *Pichia kudriavzevii*; bioenergy; biomanufacturing; bioeconomy; multistress tolerant

Introduction

A sustainable bioeconomy has recently garnered significant attention, with microbes playing a pivotal role in its development (Binati et al. 2021, Geijer et al. 2022, Ye et al. 2024). Among them, yeast has emerged as a key industrial workhorse for ethanol production due to its high yield potential, robustness in high-substrate environments, resistance to phage infections, and minimal nutritional requirements. These attributes make yeast an ideal chassis for synthetic biology, enabling the development of efficient cell factories for bio-based production across multiple sectors, including pharmaceuticals, food, commodity chemicals, and biofuels (Kitichantaropas et al. 2016, Koutinas et al. 2016, Mukherjee et al. 2017, Kong et al. 2021, Pilap et al. 2022, Sahana et al. 2024). As the demand for biomanufacturing grows, yeast-based platforms continue to drive innovation, offering scalable and versatile solutions for a sustainable bioeconomy.

Saccharomyces cerevisiae is the model yeast organism. Its genome was sequenced over two decades ago (Goffeau et al. 1996). Since then extensive functional genomics studies have continuously refined its annotation, establishing it as one of the most well-characterized eukaryotic microbes (Goffeau et al. 1996, Otero et al. 2010). Consequently, the research community has widely adopted *S. cerevisiae* as a chassis for engineering to produce a broad range of bioproducts. However the utility of *S. cerevisiae* remains constrained, particularly when lignocellulose serves as the primary substrate for bioproduct production. Although strain-to-strain variation exists, major challenges generally include limited ability to metabolize pentose sugars present in hydrolysates, low tolerance to lignocellulosic inhibitors, and sensitivity to pH and temperature fluctuation. Addressing these limitations requires not only advanced microbial engineering but also precise control of fermentation conditions to enhance strain's robustness and viability (Jetti et al. 2019, Brandt et al. 2021).

To address these challenges, certain nonconventional yeast strains with demonstrated multistress tolerance offer promising opportunities for synthetic biology and biomanufacturing. These strains can serve as alternative hosts or as reservoirs of genetic traits that confer stress tolerance, enabling the engineering of more resilient industrial strains. For instance, *Kluyveromyces marxianus* demonstrates exceptional thermotolerance, while *Pichia ku-*

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driavzevii (also known as *Issatchenkia orientalis*) thrives under extremely low pH conditions and tolerates high concentrations of furanics and aromatics derived from lignocellulosic hydrolysates (Abdel-Banat et al. 2010, Kwon et al. 2011, Mukherjee et al. 2017, Seong et al. 2017). Additionally, *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* exhibit strong resistance to osmotic stress, such as that induced by elevated glucose concentrations (Martorell et al. 2007, Branduardi et al. 2014, Mukherjee et al. 2017).

Harnessing the natural resilience of these nonconventional yeasts can enhance the robustness and efficiency of engineered microbial platforms, broadening their applicability across various industrial processes. However, our understanding of the specific metabolic pathways and regulatory mechanisms that enable these yeasts to withstand extreme conditions remains limited. This review aims to provide a comprehensive overview of the current literature, with a primary focus on *P. kudriavzevii*, a multistress-tolerant yeast in comparison to *S. cerevisiae*. We highlight its unique physiological traits, metabolic capabilities, and potential applications in industrial biotechnology, emphasizing how its inherent stress tolerance can be leveraged for biomanufacturing advancement (Table 1).

Potential of *P. kudriavzevii* as a chassis for advancing organic acid production

Pichia kudriavzevii is a multistress-tolerant yeast with significant biotechnological potential. Formerly classified as *I. orientalis* and *Candida krusei*, it has been identified in over 300 strains across diverse environments, including fermented foods, soil, and industrial wastewater (Chu et al. 2023). Recent advancements in genetic engineering, such as episomal plasmid systems and CRISPR-Cas9 genome editing, have enhanced its tractability for synthetic biology applications. Recognizing its industrial potential, the U.S. Department of Energy Center for Bioenergy and Bioproducts Innovation (CABBI) has designated *P. kudriavzevii* as a flagship strain for advancing a sustainable bioeconomy (Cao et al. 2020, Fatma et al. 2023). Its exceptional resilience under extreme conditions makes it a promising chassis for biofuel and biochemical production, particularly in lignocellulosic and organic acid fermentations (Cao et al. 2020, Suthers and Maranas 2022).

Organic acids are an important class of chemicals in the sustainable bioeconomy, serving as key precursors for a wide range of commodity chemicals and polymers. They are essential in the production of plastics, fabrics, solvents, resins, coatings, adhesives, and surfactants, among other industrial applications (Sauer et al. 2008, Singh et al. 2017, Ghai et al. 2023). The bioconversion of lignocellulose into organic acids has long been a major goal in the transition to renewable chemicals and is crucial for the large-scale deployment of a sustainable bioeconomy. A 2004 report by the U.S. Department of Energy's Office of Energy Efficiency and Renewable Energy (DOE EERE) identified the top 12 value-added chemicals derived from biomass, highlighting their economic and industrial significance (DOE EERE report). Remarkably, 9 out of these 12 chemicals are organic acids or their derivatives, reinforcing their pivotal role in replacing petroleum-based chemicals with sustainable, bio-based alternatives.

Industrial production of organic acids from lignocellulose presents significant challenges, particularly during fermentation. Lignocellulosic hydrolysates contain high concentrations of inhibitors such as acetate, furanics, and aromatics, which can hinder microbial growth and productivity. Additionally, as organic acids accumulate, the culture pH drops, further inhibiting yeast metabolism and fermentation efficiency (Thomas et al. 2002, Ferraz et al. 2023). To mitigate these issues, strategies such as inhibitor detoxification and pH adjustments using buffering agents or alkali additions are commonly employed. However, these interventions increase production costs and introduce additional impurities that complicate downstream processing (Wang et al. 2024c). Effective pH management is essential for maintaining solubility and optimizing recovery, both of which are dictated by the pKa values of the target compounds. During mid-to-late fermentation, pH is typically maintained above the pKa to keep organic acids in their soluble form, ensuring efficient microbial metabolism and product accumulation. Conversely, during the extraction and purification phase, pH is lowered below the pKa to convert the organic acids into their protonated form, improving recovery and purification efficiency. While this strategy maximizes yield, it also adds complexity to the overall production process.

Given these challenges, utilizing yeast strains such as *P. kudriavzevii*, which exhibit broad pH tolerance and resistance to lignocellulosic inhibitors, presents a promising solution. These robust strains can sustain organic acid production even as pH drops, reducing the need for costly detoxification strategies and pH adjustments (Chu et al. 2023, Dolpatcha et al. 2023). *P. kudriavzevii* has demonstrated the ability to produce succinic acid without the addition of neutralizers, owing to its high tolerance to acidic environments (Tran et al. 2023). Additionally, pH-tolerant strains have been shown to reduce byproduct formation associated with pH stress responses, such as excess glycerol accumulation and incomplete fermentation (Goold et al. 2017). By minimizing these inefficiencies, production processes can achieve higher yields, titers, and productivity, ultimately improving the economic feasibility of organic acid biosynthesis. Given these advantages, *P. kudriavzevii* is increasingly being recognized as an emerging model strain, with researchers actively exploring its potential for biomanufacturing various compounds, including ethanol, lactic acid, itaconic acid, xylonic acid, citramalic acid, and succinic acid—many of which are toxic to *S. cerevisiae* at high concentrations (Thorwall et al. 2020, Lee et al. 2022, Tran et al. 2023, Wu et al. 2023, Tan et al. 2024). Due to its innate multistress tolerance, particularly to low pH, high temperatures, osmotic stress, ethanol stress, and lignocellulosic inhibitors, *P. kudriavzevii* has the potential to enable disruptive biomanufacturing technologies, bringing closer the long-awaited goal of efficient lignocellulose-based bioprocessing.

Mechanisms of acid tolerance

While *S. cerevisiae* has been extensively studied for its pH homeostasis mechanisms, many nonmodel yeasts, such as *P. kudriavzevii*, exhibit superior low pH tolerance but remain less characterized. Understanding these mechanisms can lead to the development of robust yeast strains with enhanced industrial applications. This section explores acid tolerance mechanisms in both *S. cerevisiae* and *P. kudriavzevii*, comparing known pathways and highlighting key knowledge gaps. By identifying the genes responsible for low pH adaptation, future research can engineer more resilient yeast strains for industrial fermentation, ultimately reducing processing costs and increasing production efficiency (Chen and Nielsen 2013) (Fig. 1).

Mechanisms of acid tolerance in *S. cerevisiae*

The yeast cell wall plays an important role in low pH stress adaptation, dynamically regulating its integrity to maintain cellular stability and survival under fluctuating environmental conditions

Table 1. Overview of stress tolerance traits in *P. kudriavzevii* and their implications for industrial fermentation processes.

Phenotype	Description	Industrial relevance	References
Acid tolerance	Tolerates very low pH (as low as 1.5) and tolerates presence of weak acids (e.g. lactic, acetic, and propionic acids)	Organic acid production, fermentation of acidic substrates, and acidic fermentation processes	Isono et al. (2012), Park et al. (2018), Wu et al. (2023), Dubinkina et al. (2024)
Heat tolerance	Grows at elevated temperatures (up to 50 °C)	High-temperature fermentations, reduced cooling costs, and reduced bacterial contamination	Isono et al. (2012), Park et al. (2018), Li et al. (2021), Wang et al. (2025)
Lignocellulosic inhibitor tolerance	Tolerates toxic compounds such as furan derivatives, organic acids, and phenolics released during lignocellulosic biomass pretreatment	Bioethanol and biochemical production from lignocellulosic biomass	Mukherjee et al. (2017), Seong et al. (2017), Lee et al. (2022)
Ethanol tolerance	Withstands and ferments in high ethanol concentrations	Bioethanol production	Koutinas et al. (2016), Hoppert et al. (2022)
Osmotolerance	Grows in high sugar or salt concentrations	High-glucose fermentations in food and beverage industry, omitting desalting procedures during bioproduction	Li et al. (2021), Chu et al. (2023)

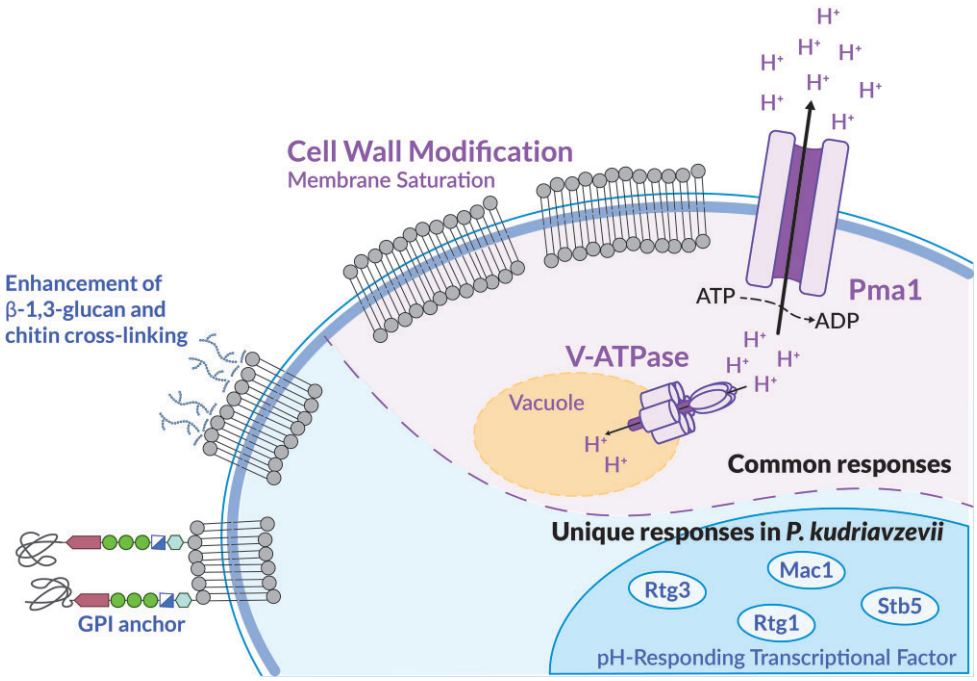

Figure 1. Mechanisms of pH tolerance in *S. cerevisiae* and *P. kudriavzevii*. This figure summarizes cellular and molecular strategies employed by *S. cerevisiae* and *P. kudriavzevii* to maintain pH homeostasis under acidic stress. In *S. cerevisiae*, key mechanisms include proton extrusion via plasma membrane H^+-ATPases (Pma1), vacuolar acidification through V-ATPases, and dynamic remodeling of the cell wall. *Pichia kudriavzevii*, which displays exceptional acid tolerance, utilizes similar core mechanisms but also exhibits distinct physiological adaptations such as increased membrane saturation, enhanced β-1,3-glucan and chitin cross-linking in the cell wall, and expression of specialized proteins like PkGAS1—a GPI-anchored protein that enhances survival at pH values below 2.0. Transcriptomic analyses further reveal regulatory contributions from transcription factors (e.g. Stb5, Mac1, and Rtg1/3), underscoring the potential of *P. kudriavzevii* as a robust microbial chassis for biomanufacturing under extreme pH conditions.

(Cabib et al. 1989, Ribeiro et al. 2022). To survive in diverse environments, *S. cerevisiae* has evolved multiple pH homeostasis mechanisms that ensure metabolic stability under acidic stress conditions.

One of the primary strategies for pH regulation in *S. cerevisiae* is proton extrusion via Pma1, a plasma membrane P2-type H^+-ATPase. Pma1 actively pumps protons (H^+) out of the cell to regulate cytosolic pH, particularly in acidic environments, where excess protons can lead to intracellular acidification and metabolic disruption. This adenosine triphos phate (ATP) dependent process prevents cytoplasmic acidification while maintaining the electrochemical gradient necessary for nutrient uptake and ion homeostasis (Meena et al. 2011).

In addition to plasma membrane proton extrusion, *S. cerevisiae* employs vacuolar acidification via V-ATPase, a vacuolar ATPase complex that sequesters excess protons into intracellular compartments (Graham et al. 2003, Forgac 2007). This system functions as an intracellular pH buffer, preventing excessive cytosolic acidification while also contributing to organelle acidification, which is essential for protein degradation, ion storage, and autophagy (Parra et al. 2014). Mutations in this pathway result in impaired stress resistance and reduced growth in acidic environ-

ments, suggesting its essential role in pH homeostasis (Deprez et al. 2021).

Mechanisms of acid tolerance in *P. kudriavzevii*

Pichia kudriavzevii has emerged as a robust microbial chassis for industrial biomanufacturing due to its remarkable tolerance to acidic conditions. While *S. cerevisiae* struggles to grow below pH 3.0, *P. kudriavzevii* thrives at pH values as low as 1.5–2.0, making it particularly attractive for organic acid production processes, where low-pH fermentation is desirable. This acid tolerance minimizes the need for neutralizers, thereby reducing downstream processing costs and enhancing process sustainability.

Although the mechanistic basis of *P. kudriavzevii*'s acid tolerance remains incompletely understood, accumulating evidence suggests that it relies on a combination of conserved and unique physiological and genetic strategies. Like *S. cerevisiae*, *P. kudriavzevii* employs proton extrusion systems to maintain intracellular pH homeostasis. Plasma membrane H^+-ATPases actively pump protons out of the cytoplasm, counteracting acidification caused by the external environment (Li et al. 2022). Although direct evidence for V-ATPase activity in *P. kudriavzevii* is not yet available, similar mechanisms characterized in other yeast species such as *S. cerevisiae* suggest that it may contribute to intracellular pH regulation (Diakov and Kane 2010).

Beyond these conserved mechanisms, *P. kudriavzevii* demonstrates several unique adaptations that contribute to its exceptional acid resilience (Ribeiro et al. 2022). These include modifications to cell wall architecture. Metabolic adjustments and transporter activities also play a role, helping to maintain redox balance and ionic homeostasis under low-pH conditions (Ribeiro et al. 2022). Understanding the full complement of these acid tolerance mechanisms—particularly those unique to *P. kudriavzevii*—will be essential for harnessing and optimizing this nonconventional yeast for industrial-scale bioprocesses. Future research into its regulatory networks, stress-responsive genes, and membrane remodeling pathways could unlock new strategies for engineering acid-tolerant microbial platforms for next-generation biomanufacturing.

Membrane and cell wall adaptations

One major difference between *P. kudriavzevii* and *S. cerevisiae* is its membrane lipid composition. Studies indicated that *P. kudriavzevii* has a higher ratio of saturated fatty acids, which may enhance membrane rigidity and reduce acid permeability, providing greater resistance to acid-induced stress (Gao et al. 2021).

In addition, cell wall modifications play a critical role in acid resistance. *Pichia kudriavzevii* exhibits increased β-1,3-glucan and chitin cross-linking, which strengthens the cell wall and reduces its permeability to organic acids (Ribeiro et al. 2022). Transcriptomic and proteomic studies of *P. kudriavzevii* under acetic acid stress reveal the upregulation of stress response pathways, including genes related to cell wall remodeling, oxidative stress response, and membrane transporters (Li et al. 2022). Specifically, the upregulation of MPG1, a GDP-mannose pyrophosphorylase, suggests a role in maintaining cell wall integrity (CWI) under acid stress, a critical factor for survival in extreme pH conditions (Li et al. 2022).

PkGAS1: a key determinant of acid tolerance

An important discovery in acid tolerance of *P. kudriavzevii* is PkGAS1, a glycosylphosphatidylinositol (GPI)-anchored cell wall protein (CWP). Overexpression of PkGAS1 in *S. cerevisiae* significantly enhances acid tolerance, conferring resistance to multiple acids, including sulfuric, hydrochloric, formic, acetic, and lactic acids, particularly at pH values below 2.4 (Wada et al. 2020). The proposed mechanism of PkGAS1 involves reinforcement of the cell wall, improving structural stability, and reducing acid-induced cellular damage. Additionally, PkGAS1-expressing *S. cerevisiae* strains show improved ethanol fermentation performance under acidic conditions, suggesting its industrial potential in bioethanol and organic acid production (Wada et al. 2020).

PkGAS1 shares significant homology with other pH adaptation proteins. It exhibits 60% identity with *S. cerevisiae* Gas1p, a GPI-anchored protein involved in cell wall assembly, and 58%–59% homology with *Candida albicans* Phr1 and Phr2, which are implicated in pH adaptation and cell wall stability (Matsushika et al. 2016). PkGAS1 expression is pH-dependent, with its expression levels increasing as pH decreases from 4.0 to 2.0, further supporting its role in acid stress adaptation (Matsushika et al. 2016). Site-directed mutagenesis studies have identified two conserved glutamate residues (E161 and E262) as critical for cell morphology and resistance to low pH and salt stress, suggesting their essential role in the protein's structural or functional stability (Matsushika et al. 2016).

Regulatory networks involved in acid tolerance

Recent transcriptomic analysis of *P. kudriavzevii* under extreme low pH conditions (pH 1.5) have provided deeper insights into additional regulatory mechanisms contributing to acid tolerance (Dubinkina et al. 2024). Comparative studies between pH-tolerant and pH-susceptible strains identified key genes involved in energy metabolism, translation-related processes, and the CWI pathways (Dubinkina et al. 2024). Several transcription factors, including Stb5, Mac1, and Rtg1/Rtg3, were implicated in low pH adaptation, suggesting their importance in maintaining pH homeostasis (Dubinkina et al. 2024).

Mechanisms of heat tolerance

Heat tolerance in yeast is governed by a complex regulatory network of genetic, molecular, and physiological processes that collectively enable cells to withstand both acute and prolonged heat stress. In the short term, cytoprotective mechanisms primarily involve the transient expression of heat shock proteins (HSPs), which prevent the misfolding and aggregation of cellular proteins (Jarolim et al. 2013, Kyriakou et al. 2023). In contrast, prolonged heat stress triggers more extensive transcriptional reprogramming, promoting cellular recovery and metabolic rebalancing (Jarolim et al. 2013, Riles and Fay 2019, Gan et al. 2021) (Fig. 2).

Heat tolerance in *S. cerevisiae*

Saccharomyces cerevisiae serves as a primary model for thermotolerance research. It relies on a core network of transcriptional factors, including Sin3p, Srb2p, and Mig1p, to regulate the expression of stress-related genes in a hierarchical manner (Gan et al. 2021). The deletion of *SIN3* and *SRB2* significantly reduces high-temperature fermentation capacity, underscoring their crucial role in maintaining physiological homeostasis, whereas *MIG1* deletion has a comparatively minor effect.

Thermotolerant strains typically exhibit sustained expression of HSPs (e.g. *HSP70*, *HSP90*, and *HSP104*) and trehalose biosynthetic genes (*NTH1*, *TPS1*, *TPS2*, *ELM1*, and *SAK1*), which together protect cellular proteins from heat-induced denaturation (Kitichantaropas et al. 2016, Asada et al. 2022, Wang et al. 2022, Kyriakou

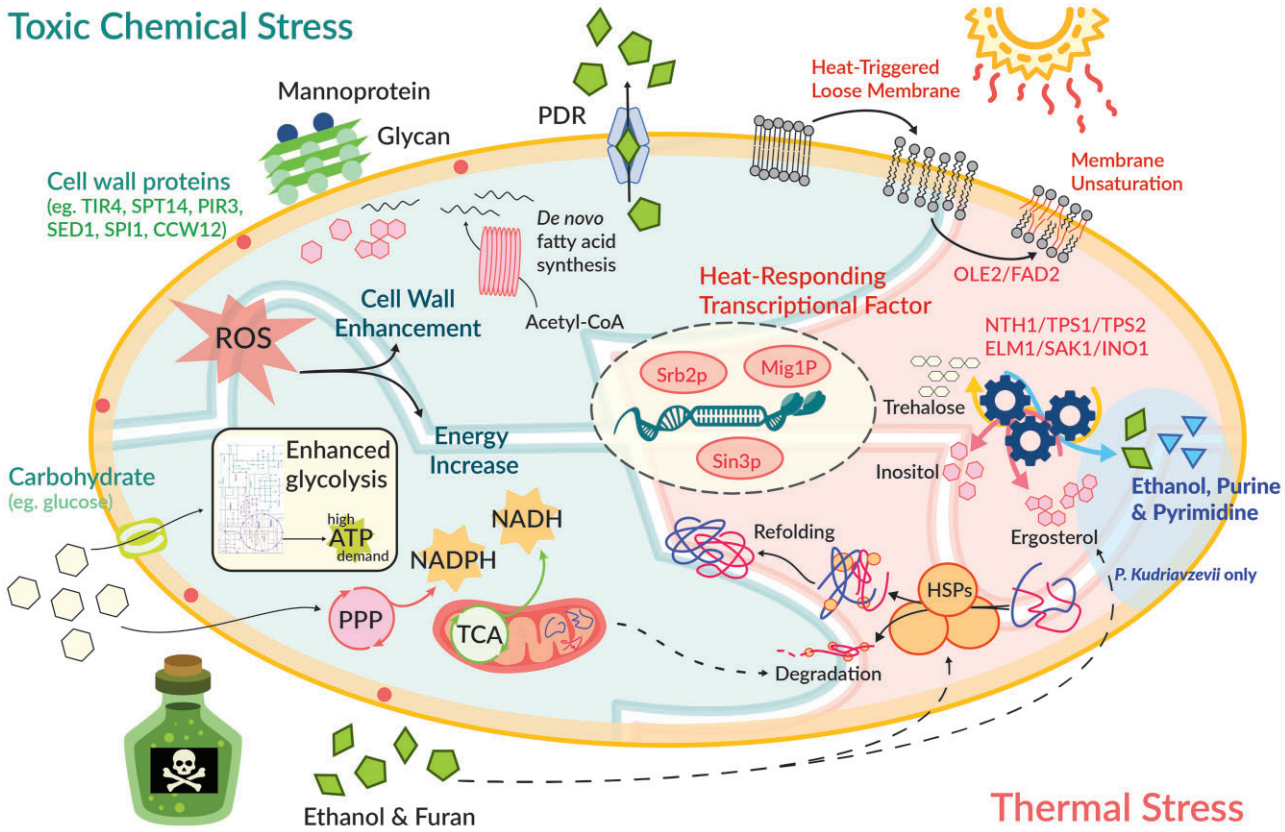

Figure 2. Mechanisms of toxic chemical and heat tolerance in *S. cerevisiae* and *P. kudriavzevii*. Toxic chemical tolerance: toxic compounds, including lignocellulosic inhibitors and ethanol, trigger protective responses in both yeasts. *Saccharomyces cerevisiae* strengthens its cell wall and membrane, activates antioxidant defenses (e.g. thioredoxins), detoxifies compounds through efflux transporters, and stabilizes proteins via HSPs and trehalose. Ethanol stress induces cross-protective mechanisms involving energy metabolism and membrane composition. *Pichia kudriavzevii* demonstrates higher tolerance through enhanced aldehyde detoxification, elevated NAD(P)H levels, robust ergosterol biosynthesis, and strong protein quality control systems. It also activates nutrient transport and sporulation pathways, suggesting broader stress adaptation potential for industrial applications. Heat tolerance: *S. cerevisiae* relies on transcription factors (e.g. SIN3 and SRB2), HSPs (HSP70, HSP90, and HSP104), trehalose biosynthesis, and membrane remodeling to protect cellular components and maintain homeostasis. It increases unsaturated fatty acid synthesis and glycolytic activity to support energy demands under heat stress. *Pichia kudriavzevii* shares some of these mechanisms but differs in its response to acute stress—suppressing trehalose and glycogen biosynthesis while upregulating ethanol production pathways. These distinct adaptations support its use in high-temperature fermentations.

et al. 2023). In addition, adjusting cell membrane composition, particularly phospholipid saturation and sterol content, plays a vital role in heat tolerance (Henderson et al. 2013, Caspeta et al. 2014). Under thermal stress, upregulation of desaturase genes (*OLE2* and *FAD2*), enhances the production of unsaturated fatty acids, improving membrane fluidity and integrity, which is essential for protein mobility and protection against heat-induced damage (Degreif et al. 2017, Lu et al. 2022, Wang et al. 2024a).

These protective mechanisms impose an increased energy demand, leading to a metabolic shift toward glycolysis to meet ATP requirements (Kitichantaropas et al. 2016, Gan et al. 2021, Wang et al. 2022). Additionally, transposon activity and mitochondrial stress responses may further contribute to the adaptive evolution of thermotolerance (Kaur et al. 2021, Takagi 2021, Lu et al. 2022).

Heat tolerance in *P. kudriavzevii*

Unlike *S. cerevisiae*, the adaptive evolution of nonmodel yeast species under heat stress remains largely unexplored. Preliminary studies suggest that under long-term heat exposure, *P. kudriavzevii* employs similar thermoprotective strategies, including the upregulation of HSPs (*hsp90* and *ssq1*), enhanced glycolytic flux, and membrane remodeling to maintain cellular integrity (Kitagawa et al. 2010, Seong et al. 2017, Li et al. 2018, 2021). The accumulation of protective metabolites such as trehalose and glycerol further contributes to cellular stability under prolonged heat stress.

However, *P. kudriavzevii* exhibits distinct thermotolerance responses compared to model yeasts. Under acute heat shock, genes involved in trehalose (*nthu1606*, *nth1572*, and *ggs1*) and glycogen (*gsk3*) biosynthesis are downregulated, suggesting a stress adaptation strategy different from *S. cerevisiae* (Chamnipa et al. 2018). Interestingly, ethanol biosynthesis genes (*adh1* and *adh3*) are consistently upregulated under both acute and prolonged heat stress, indicating a metabolic shift that favors energy generation in response to thermal stress (Chamnipa et al. 2018).

These unique thermoresponse mechanisms highlight the potential of *P. kudriavzevii* for high-temperature bioethanol production. However, the underlying molecular mechanisms and transcriptional regulatory networks governing thermotolerance in nonmodel yeasts remain poorly understood. Further research is needed to fully harness their potential for industrial applications, particularly in the high-temperature fermentation process.

Mechanisms for tolerance to toxic compounds

Yeasts used in industrial processes frequently encounter toxic compounds, primarily lignocellulosic inhibitors. These stressors can impair cellular function, reduce metabolic efficiency, and hinder fermentation performance. To be industrially viable, yeast strains must develop strategies to tolerate diverse categories of toxic compounds (Fig. 2).

Lignocellulosic inhibitors

Lignocellulosic biomass, the most abundant biopolymer on Earth, is a valuable renewable resource composed mainly of cellulose, hemicellulose, and lignin (Yuan et al. 2017). Pretreatment of this biomass generates lignocellulosic hydrolysates, which serve as feedstock for microbial fermentation in the production of biofuels and industrial chemicals (Wang et al. 2024a). However, pretreatment processes release various inhibitory compounds that challenge yeast fermentation performance, and their compositions change based on the raw materials and pretreatment procedures. These inhibitors include furan derivatives, such as 5-hydroxymethyl-2-furfural (HMF) and furfural, organic acids, such as formic, acetic and levulinic acids, as well as phenolic compounds, such as phenol, vanillin, 4-hydroxybenzaldehyde, and syringaldehyde (Zhang et al. 2012, Thompson et al. 2016, Hoppert et al. 2022, Pilap et al. 2022, Zhao et al. 2024). For yeast strains to be industrially viable, they must not only tolerate these inhibitors but also withstand high concentrations of their own metabolic products such as ethanol, isobutanol, and succinic acid.

Tolerance mechanisms for lignocellulosic inhibitors in *S. cerevisiae*

Saccharomyces cerevisiae employs employs a multifaceted defense system to withstand toxic lignocellulosic inhibitors, including cell wall remodeling, redox homeostasis, protein repair, and active detoxification via transporters (Kong et al. 2021, Ribeiro et al. 2022). The cell wall and membrane serve as the first line of defense, acting as physical barriers against external stress. Once inhibitors penetrate the cells, *S. cerevisiae* utilizes efflux transporters to actively export these compounds, either in their original form or as less toxic derivatives resulting from intracellular catabolism (Unrean et al. 2018, Liu and Ma 2020, Goud et al. 2022). To further mitigate cellular damages, *S. cerevisiae* activates redox-balancing pathways to neutralize reactive oxygen species (ROS) and correct cofactor imbalances associated with detoxification (Pahlman et al. 2001, Wolak et al. 2014, Hopkins and Neumann 2019, Liu et al. 2019, Liu and Ma 2020, Goud et al. 2022). In addition, cellular repair mechanisms are critical for maintaining protein integrity under chemical stress (Ding et al. 2012, Wang et al. 2013, Kitichantaropas et al. 2016, Unrean et al. 2018, Liu and Ma 2020, Goud et al. 2022).

Physical barriers and elimination

The yeast cell wall and membrane serve as the first line of defense against toxic compounds. *Saccharomyces cerevisiae* modulates its cell wall composition (β-1,3- and β-1,6-glucans, chitins, mannans, and cross-linking proteins) in response to stress by increasing the expression of genes involved in cell wall biosynthesis, rigidity, and maintenance (Levin 2011, Liu and Ma 2020, Ribeiro et al. 2022). Notably, inositol-3-phosphate synthase (Ino1p), responsible for phospholipids biosynthesis, is significantly upregulated under stress conditions. Additionally, genes encoding GPI-anchored CWPs (*TIR4*, *SPT14*, *PIR3*, *SED1*, and *SPI1*) exhibit elevated transcription, reinforcing the cell wall (Levin 2011, Unrean et al. 2018, Liu and Ma 2020). Trehalose biosynthetic genes are also upregulated, stabilizing cellular membranes and preventing damage caused by toxic compounds (Unrean et al. 2018). These responses indicate that *S. cerevisiae* is actively remodeling its cell wall and membrane to mitigate the effects of lignocellulosic inhibitors.

Redox homeostasis and detoxification

Furanic and phenolic compounds induce ROS, triggering oxidative stress. *Saccharomyces cerevisiae* mitigates this through enzymatic and nonenzymatic pathways, scavenging ROS to protect cellular components (Unrean et al. 2018).

Saccharomyces cerevisiae metabolizes aldehydes into less toxic compounds using nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reductases. However, this process depletes NADPH, creating a redox imbalance (Unrean et al. 2018, Liu et al. 2019). The pentose phosphate pathway is upregulated, with increased expression of glucose-6-phosphate dehydrogenase (*ZWF1*) and 6-phosphogluconate dehydrogenase (*GND1*), both critical for NADPH regulation (Liu et al. 2019). Conversely, glycerol-3-phosphate phosphatase (*GPP1*), involved in glycerol biosynthesis is downregulated, reducing unnecessary NADPH consumption (Pahlman et al. 2001, Unrean et al. 2018).

Thioredoxin (*TRX1*) and glutaredoxin are significantly upregulated, neutralizing oxidative stress (Hopkins and Neumann 2019, Liu and Ma 2020). *TRX1* also acts as a cofactor for thioredoxin peroxidase, an antioxidant involved in detoxification of ROS (Unrean et al. 2018). Thiamine (vitamin B1) is essential for maintaining redox homeostasis. The upregulation of thiamine biosynthesis (*THI13* and *THI14*) and metabolism genes (*THI2*, *THI3*, *THI13*, *THI20*, *THI22*, and *THI74*) suggests that thiamine plays a crucial role in coping with furfural stress (Wolak et al. 2014, Goud et al. 2022).

Protein stability and repair

Lignocellulosic inhibitors can cause intracellular acidification, leading to protein unfolding, misfolding, denaturation, and degradation (Unrean et al. 2018). For example, HSPs, such as *HSP12*, mitigate protein degradation caused by stress (Ding et al. 2012, Unrean et al. 2018, Goud et al. 2022). Trehalose accumulation stabilizes proteins, preventing aggregation and denaturation under furfural stress (Wang et al. 2013, Kitichantaropas et al. 2016, Unrean et al. 2018). Genes involved in sulfur amino acid biosynthesis are also upregulated, contributing to amino acid and nucleotide biosynthesis, which support cellular growth under stress conditions (Liu and Ma 2020).

Transporter-mediated detoxification

Efflux transporters actively export toxic compounds from the cells. Pleiotropic drug resistance transporters and their associated transcription factors are upregulated, indicating that *S. cerevisiae* actively pumps out toxic chemicals (Unrean et al. 2018, Liu and Ma 2020). Hexose transporters (*HXT6* and *HXT7*), and thiamine transporter (*THI7*) are significantly upregulated under stress, supporting both detoxification and carbon metabolism (Goud et al. 2022)

Tolerance mechanisms in in *P. kudriavzevii*

Studies suggest that *P. kudriavzevii* exhibit greater tolerance of furanic and phenolic compounds than *S. cerevisiae* (Ding et al. 2012, Isono et al. 2012, Mukherjee et al. 2017, Seong et al. 2017, Lee et al. 2022) However, the specific molecular mechanisms underlying this tolerance remain poorly understood. Quantitative re-

verse transcription polymerase chain reaction (RT-qPCR) analysis indicates the upregulation of aldehyde dehydrogenase family proteins, suggesting an enhanced ability to metabolize toxic compounds (Akita and Matsushika 2024). Observations during ethanol production from lignocellulosic hydrolysates indicate that intracellular NADH/NADPH levels are elevated, likely to support aldehyde detoxification (Yuan et al. 2017). The structural composition of the *P. kudriavzevii* cell wall and membrane is similar to *S. cerevisiae*, implying that its physical defence mechanisms may be conserved (Suthers et al. 2020). While *P. kudriavzevii* demonstrates superior tolerance to lignocellulosic inhibitors, further research is needed to elucidate the regulatory networks responsible for its resilience. A deeper understanding of its stress response mechanisms could enhance its potential as an industrial fermentation host.

Mechanism for ethanol tolerance

Ethanol is one of the major biochemicals produced by yeast and plays a key role in bioethanol production for fuel, as well as in the brewing and alcoholic fermentation industries (Thorwall et al. 2020). However, at high concentrations, ethanol becomes toxic to yeast cells, reducing productivity and limiting titers in fermentation processes. Ethanol stress imposes membrane, protein, and metabolic challenges on yeast cells. *Saccharomyces cerevisiae* responds by reinforcing its cell wall and membrane, stabilizing proteins via trehalose and HSPs, and activating detoxification pathways. However, *P. kudriavzevii* exhibits greater ethanol tolerance due to its enhanced ergosterol biosynthesis, superior protein refolding capacity, and unique metabolic adaptations. Understanding these mechanisms can guide the engineering of more robust yeast strains for industrial fermentation (Figs 2 and 3).

Ethanol stress response in *S. cerevisiae*

Saccharomyces cerevisiae counters ethanol stress through structural and metabolic adaptations. Ethanol disrupts membrane integrity, promoting reinforcement of the cell wall and membrane via upregulation of CWI pathway genes, sterol biosynthesis, and cell wall-associated proteins (Ribeiro et al. 2022).To maintain protein stability, the yeast accumulates trehalose and induces HSPs. Detoxification involves transporter activity and vesicle trafficking. Ethanol also triggers cross-tolerance responses by activating genes involved in redox balance, energy metabolism, and cofactor regeneration.

Cell wall and membrane reinforcement

Ethanol disrupts the cell wall and membrane, reducing stiffness and increasing permeability. Its lipophilic and amphiphilic properties perturb membrane lipids, alter membrane organization, and compromise the transmembrane electrochemical potential (Ribeiro et al. 2022). To counteract these effects, these cells reinforce their cell walls and membranes by upregulating genes involved in CWI, including those encoding CWPs and components of CWI pathway (Jung and Levin 1999, Sanz et al. 2017).

Additionally, studies have shown that genes involved in the biosynthesis of mannoproteins and β-glucans, as well as CCW12, which enhances cell wall stability and stress resistance, are upregulated (Schiavone et al. 2016, Kong et al. 2021). GPI-anchored proteins link the cell wall to the membrane and are destabilized under ethanol stress (Jung and Levin 1999, Schiavone et al. 2016).

Genes involved in fatty acid and sterol biosynthesis are also upregulated to stabilize the membrane integrity. While genes encoding ergosterol and inositol biosynthesis are not typically upregulated, mutant studies show that deficiencies in these metabolites reduce ethanol tolerance, highlighting their importance (Furukawa et al. 2004). Ergosterol is essential as a cytoplasmic membrane protectant, maintaining the membrane structure and alleviating the ethanol-induced dissipation of the transmembrane electrochemical potential. Inositol containing membrane proteins may impact membrane permeability and play a role in ion homeostasis in the cytoplasm. Furthermore, various HSPs and transcription factors involved in cell wall organization are upregulated, further reinforcing cellular structures (Kitichantaropas et al. 2016, Zhao et al. 2017, Kyriakou et al. 2023)

Protein stability and protection

Ethanol causes protein denaturation and aggregation, disrupting enzymatic activity and cellular function. Yeast employs several mechanisms to protect and stabilize proteins. Trehalose acts as molecular chaperone, protecting membrane proteins from misfolding and aggregation. Genes involved in trehalose biosynthesis are upregulated under ethanol stress (Alexandre et al. 2001, Kitichantaropas et al. 2016). Trehalose also influences other metabolic pathways to effectively accumulate ATP in cells. Interestingly, genes involved in trehalose degradation are also upregulated, suggesting that maintaining an optimal trehalose concentration is necessary for ethanol stress adaptation (Alexandre et al. 2001). Proteins involved in protein folding and stabilization, such as HSP26 and SSA3, are significantly upregulated (Alexandre et al. 2001).

Transporter-mediated detoxification

Ethanol tolerance at high temperatures is associated with the upregulation of *PSD1*, a mitochondrial inner membrane protein, and *SEC24*, a vesicle formation component involved in ER-to-Golgi transport (Riles and Fay 2019). Vesicle trafficking systems likely facilitate the removal of denatured proteins from organelles into the cytoplasm for degradation. Additionally, genes involved in trehalose biosynthesis (*TPS1* and *TPS2*) and plasma membrane ion transport regulation are overexpressed under ethanol stress (Goud et al. 2022).

Cross-tolerance mechanisms

Ethanol stress induces osmotic stress, redox imbalance, protein denaturation, and heat stress, triggering a broad cross-tolerance response (Alexandre et al. 2001, Goud et al. 2022). For example, genes involved in osmotic and redox balance such as glyceraldehyde-3-phosphate (*TDH1*, *TDH2*, and *TDH3*) and pyruvate kinase (*CDC19*) are highly upregulated. Glucose-sensing genes (*SNF1*, *SSA3*, *HXK1*, *SNF1*, *STR3*, *ATG1*, and *HXT7*) and other genes involved in NADPH/NADH regeneration and glycolysis are activated, indicating that yeast prioritizes ATP regeneration and cofactor balance under ethanol stress (Alexandre et al. 2001, Goud et al. 2022).

Ethanol stress response in *P. kudriavzevii*

Compared to *S. cerevisiae*, *P. kudriavzevii* exhibits greater ethanol tolerance, outperforming *S. cerevisiae* in fermenting lignocellulosic hydrolysates at elevated temperatures, higher salinity, increased osmolarity, lower pH, and in the presence of lignocellulosic inhibitors (Isono et al. 2012, Kwon et al. 2013, Koutinas et al. 2016, Mukherjee et al. 2017, Seong et al. 2017, Yuan et al. 2017, Chamnipa et al. 2018, Li et al. 2018, Lee et al. 2022, Pilap et al. 2022, Sahana et al. 2024)

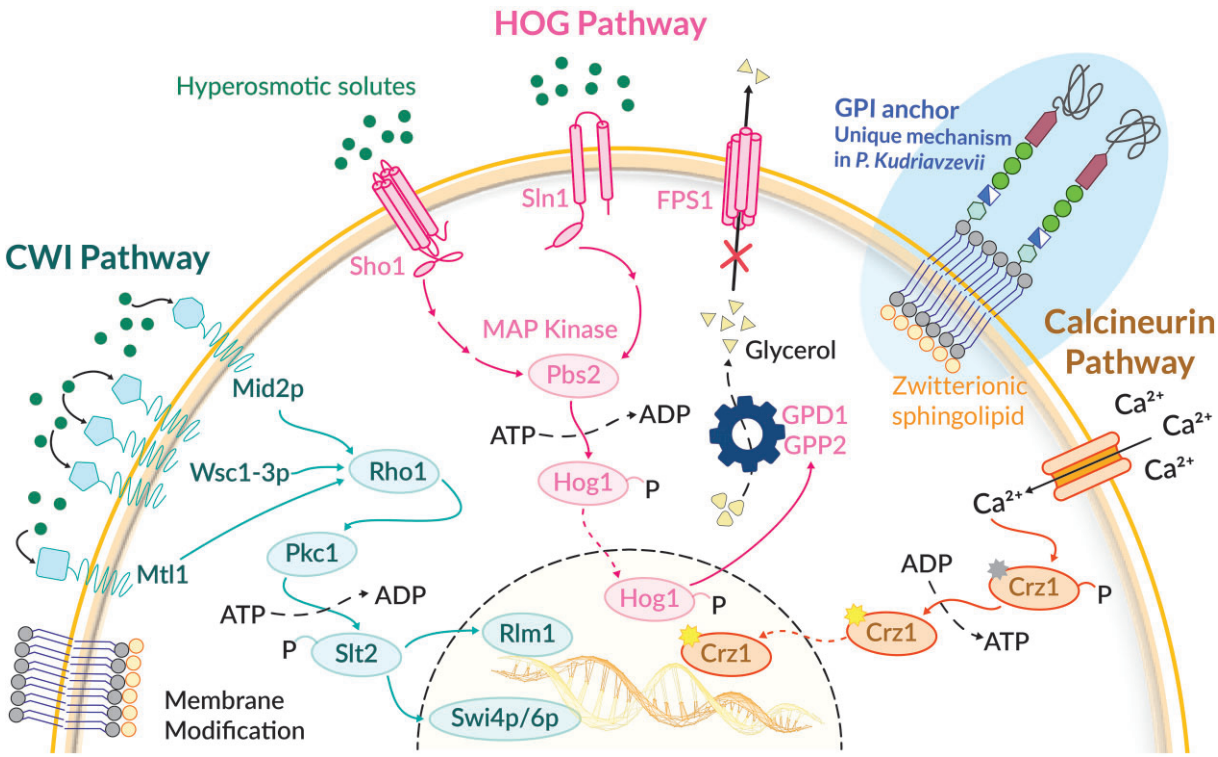

Figure 3. Mechanisms of osmotic stress tolerance in *S. cerevisiae* and *P. kudriavzevii*. In *S. cerevisiae*, the HOG pathway senses osmotic changes via Sln1p and Sho1p branches, activating Hog1p to induce glycerol biosynthesis (*GPD1* and *GPP2*) and reduce glycerol loss through Fps1p. The CWI pathway, through sensors like Wsc1p and Mid2p, promotes cell wall remodeling via Slt2p. Additional support comes from the calcineurin-Crz1p system, cAMP-PKA signaling, and membrane lipid remodeling (e.g. Elo2p, Cds1p, and Cho1p), which maintain membrane integrity and ion balance. *Pichia kudriavzevii* exhibits high osmotic tolerance (up to 50% sugars, 1.5 M salts). Although less characterized, it likely uses a conserved HOG pathway, as shown by glycerol accumulation and upregulation of HOG genes in stress-adapted strains. This pathway also contributes to cross-protection against heat and acid stress. The CWI pathway may also play a role, as overexpression of *PkGAS1* enhances salt tolerance more effectively than its *S. cerevisiae* homolog, highlighting functional divergence and superior stress adaptation. The yellow star icon indicates the active form of Crz1p, which translocates into the nucleus to activate or repress the expression of its target genes.

Membrane composition and stability

Transcriptomic analysis shows that genes involved in ergosterol biosynthesis (*ERG2*, *ERG3*, and *ERG27*) in *P. kudriavzevii* under ethanol stress, whereas similar upregulation is absent in *S. cerevisiae* (Miao et al. 2018). Ergosterol synthesis and accumulation play a key role in maintaining membrane fluidity and ethanol resistance (Miao et al. 2018). Both trehalose and glycogen biosynthesis are upregulated under ethanol stress, contributing to membrane stabilization and energy storage (Miao et al. 2018). Moreover, genes and transcription factors involved in tolerance of other stressors, such as low pH and heat, are also upregulated in the presence of ethanol. Furthermore, genes involved in protein folding and refolding, and associated chaperones, are upregulated, similar to *S. cerevisiae*.

Protein stability and refolding mechanisms

Like *S. cerevisiae*, *P. kudriavzevii* upregulates genes encoding HSPs and chaperones to counteract ethanol-induced protein misfolding. Transcriptome shows that HSP binding proteins and cochaperones (*STI1*, *AHA1*, *SSE1*, *MAS5*, *FES1*, and *SIS1*) are upregulated in response to ethanol stress assisting in protein stabilization. HSP proteins (*HSP42*, *HSP78*, and *HSP104*) are upregulated to process misfolded and aggregated proteins. Genes encoding ubiquitin-protein ligases (*UBP16*, *BUL2*, *TOM1*, *HUL4*, *BRE1*, and *CUE*) are upregulated, facilitating the degradation of misfolded proteins (Glover and Lindquist 1998, Miao et al. 2018).

Nutrient transport and pseudo-starvation response

Ethanol stress in *P. kudriavzevii* induces a response similar to nitrogen starvation in *S. cerevisiae*. Transcriptome data show upregulation of genes involved in transmembrane transport, meiosis associated-genes, genes responsible for pseudo-hyphal growth, and sporulation, suggesting that ethanol triggers a nutrient-limiting response in *P. kudriavzevii* (Miao et al. 2018). Unlike *S. cerevisiae*, *P. kudriavzevii* accumulates higher levels of unsaturated fatty acids and upregulates purine and pyrimidine biosynthesis, which may enhance its ability to withstand ethanol induced stress (Seong et al. 2017).

Mechanisms of osmotic stress tolerance

Osmotic stress is one of the most prevalent challenges yeast species encounter in both natural environments and industrial processes, particularly in food and bioproduct production (Hohmann 2002, Saito and Posas 2012, Steensels et al. 2014, Ribeiro et al. 2022). Hyperosmotic stress typically arises at the beginning of fermentation due to high initial sugar concentrations and can persist as salts accumulate during the fermentation process (Saito and Posas 2012, Ribeiro et al. 2022, Chen et al. 2024). Under these conditions, elevated extracellular solute concentrations cause water efflux from the cell, leading to cellular shrinkage, impaired metabolism, and reduced viability (Hohmann 2002, Saito and Posas 2012, Chen et al. 2024). To withstand these conditions, yeast species have evolved a range of adaptive mechanisms,

with the high osmolarity glycerol (HOG) pathway (Hohmann 2002, Chen et al. 2024) and the CWI pathway playing central roles in osmoadaptation (Ribeiro et al. 2022, Chen et al. 2024) (Fig. 3).

Osmotic stress tolerance in *S. cerevisiae*

Saccharomyces cerevisiae employs a complex network of signaling pathways and adaptive responses to survive osmotic stress, which commonly arises in industrial fermentations due to high sugar concentrations and salt accumulation. Central to this response is the HOG pathway, which regulates intracellular glycerol levels to maintain osmotic balance. Complementary systems, such as the CWI pathway and additional signaling mechanisms including calcineurin and cAMP-PKA pathways, further support cell wall remodeling, ion homeostasis, and membrane integrity. Together, these coordinated responses enable *S. cerevisiae* to adapt and maintain viability under hyperosmotic conditions.

HOG pathway

The HOG pathway is a key regulatory system that helps yeast maintain osmotic balance by accumulating compatible solutes, primarily glycerol. In *S. cerevisiae*, osmotic stress is sensed by two upstream branches—the Sln1p and Sho1p sensor pathways—which converge at the mitogen-activated protein (MAP) kinase Pbs2p (Van Wuytswinkel et al. 2000, Hohmann 2002, 2009). Activated Pbs2p phosphorylates Hog1p, a MAP kinase that translocates to the nucleus to regulate stress-responsive transcription factors, leading to the upregulation of glycerol biosynthesis genes (*GPD1* and *GPP2*) while simultaneously closing the glycerol channel Fps1p, to prevent glycerol leakage (Hohmann 2002, 2009). This ensures intracellular glycerol accumulation, restoring osmotic balance and protecting the cells from dehydration. Additionally, the HOG pathway downregulates ergosterol biosynthesis genes resulting in lower ergosterol content in the plasma membrane (Montañés et al. 2011). Reduced ergosterol levels may help prevent plasma membrane rupture during osmotic stress and maintain cellular sodium homeostasis under salt-induced hyperosmotic conditions (Montañés et al. 2011, Sokolov et al. 2022).

CWI pathway

In addition to osmolyte accumulation, osmotic stress induces mechanical stress on the yeast cell wall due to rapid changes in cell volume. The CWI pathway helps maintain cell wall elasticity and integrity under these conditions. In *S. cerevisiae*, plasma membrane sensors (Wsc1-3p, Mid2p, and Mtl1p) detect mechanical stress and activate a signaling cascade through the GTPase Rho1p and protein kinase Pkc1p (Ribeiro et al. 2022, Chen et al. 2024). This ultimately activates MAP kinase Slt2p, which regulates genes involved in cell wall remodeling through transcription factors such as Rlm1p, Swi4p, and Swi6p (Ribeiro et al. 2022, Chen et al. 2024). Interestingly, the CWI and HOG pathways are interconnected, as the CWI sensors Mid2p and Wsc1p detect changes in turgor pressure caused by glycerol accumulation, facilitating coordinated responses to osmotic stress (García-Rodríguez et al. 2005).

Additional stress response pathways

In addition to the HOG and CWI pathways, *S. cerevisiae* employs additional regulatory mechanisms to enhance osmotic stress tolerance. Calcineurin, a calcium- and calmodulin-dependent phosphatase, is activated by hyperosmotic-stress-induced calcium influx, leading to the dephosphorylation of Crz1p, a transcription factor regulating ion homeostasis and CWI (Panadero et al. 2007, Zuo et al. 2024). cAMP-PKA pathway also contributes to osmoadaptation. Environmental stimuli elevate cAMP levels, activating protein kinase A (PKA) catalytic subunits (Tpk1p, Tpk2p, and Tpk3p), which modulate gene expression and metabolism to support general stress responses (Ribeiro et al. 2022, Galello et al. 2024). While the HOG pathway directly addresses osmotic stress, the cAMP-PKA pathway provides broader adaptive support. Moreover, a recent adaptive laboratory evolution experiment revealed that upregulation of the sphingolipid acyl chain elongase Elo2p increases levels of very-long-chain fatty acids and complex sphingolipids, thus enhancing membrane integrity under osmotic stress (Zhu et al. 2020). Furthermore, overexpressing the phosphatidate cytidylyltransferase Cds1p and the phosphatidylserine synthase Cho1p lowers the anionic-to-zwitterionic phospholipid ratio and was found to enhance salt tolerance in *S. cerevisiae* (Yin et al. 2020).

Osmotic stress tolerance in *P. kudriavzevii*

Pichia kudriavzevii also exhibits exceptional osmotic tolerance with reported limits of 48% (w/v) glucose, 50% (w/v) fructose, 50% (w/v) sorbitol, 1.0 M NaCl, and 1.5 M KCl (Mukherjee et al. 2017). Although direct evidence supporting the involvement of the HOG pathway in the osmotic stress response in *P. kudriavzevii* remains limited, the substantial accumulation of glycerol observed in *P. kudriavzevii* when grown in media with high glucose concentrations strongly suggests that the HOG pathway likely plays a significant role in its osmotic tolerance (Chu et al. 2023).

Interestingly, besides its potential role in osmotic tolerance, the HOG pathway has also been reported to contribute to acid and heat tolerance accompanied by increased osmotic stress resistance in *P. kudriavzevii* (Dolpatcha et al. 2023, Akita and Matsushika 2023, Li et al. 2021). Adaptive laboratory evolution experiments indicated that the evolved acetic acid-tolerant *P. kudriavzevii* strains also exhibited enhanced osmotic tolerance with elevated expression of HOG pathway genes compared to their parental unevolved strains (Dolpatcha et al. 2023, 2025). Similarly, the cross-protection against heat stress induced by salt treatment may also involve activation of the HOG pathway, as evidenced by the increased glycerol accumulation observed in *P. kudriavzevii* (Li et al. 2021). Overall, these studies indicate that the HOG pathway and its downstream stress-responsive genes may serve as a general protective mechanism enabling *P. kudriavzevii* to tolerate diverse stress conditions.

Beyond the HOG pathway, the CWI pathway has been suggested to play a role in salt tolerance in *P. kudriavzevii*. The cell wall GPI-anchored protein PkGas1p found in *P. kudriavzevii* has been shown to increase tolerance to Na_2SO_4 under low-pH conditions (Matsushika et al. 2016). Surprisingly, although the *S. cerevisiae* homolog *ScGAS1* is salt-responsive, overexpressing *ScGAS1* does not enhance salt tolerance as *PkGAS1* does (Matsushika et al. 2017), suggesting functional divergence between these two homologs.

Conclusion

Both *S. cerevisiae* and *P. kudriavzevii* are frequently exposed to various stressors, including acid stress, heat, high osmolarity, and toxic compounds in industrial and research settings. These yeasts employ common strategies to enhance stress tolerance, such as modifying their cell wall and membrane composition, reinforcing cell wall structures, and expressing genes that improve membrane integrity to prevent the entry of harmful molecules. Additionally, genes involved in ion and redox homeostasis, membrane

transport, heat shock response, cochaperone activity, transcriptional regulation, and stress signaling pathways are upregulated in response to environmental stress. Notably, both species exhibit cross-tolerance, where cellular pathways activated by one type of stress (e.g. ethanol stress) confer resilience to other stressors, such as osmotic, acid, and heat stress.

Despite its superior multistress tolerance, *P. kudriavzevii* remains relatively understudied compared to *S. cerevisiae*. As a result, much of our current understanding of yeast stress responses is derived from *S. cerevisiae* research. Further investigating into *P. kudriavzevii* and other nonconventional yeasts could provide valuable mechanical insights, expanding their potential for industrial applications. To address current knowledge gaps, multiomics approaches—including transcriptomics, proteomics, and metabolomics—can be employed under defined stress conditions to capture comprehensive molecular responses. When integrated with genetic manipulation and phenotypic assays, these approaches facilitate the identification and functional validation of key genes and pathways involved in stress tolerance. Furthermore, comparative multiomics studies between *S. cerevisiae* and *P. kudriavzevii* may reveal unique adaptations that underlie its exceptional resilience. Exploring the genetic and physiological basis of stress tolerance in *P. kudriavzevii* could not only optimize its use in bioproduction but also uncover novel stress adaptation mechanisms applicable to other multistress-tolerant yeasts. This knowledge could drive the development of more resilient microbial cell factories, supporting a sustainable bioeconomy and enhancing the robustness of industrial bioprocesses.

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