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Recent advances in genetic engineering and chemical production in yeast species

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Abstract

Yeasts have emerged as well-suited microbial cell factory for the sustainable production of biofuels, organic acids, terpenoids, and specialty chemicals. This ability is bolstered by advances in genetic engineering tools, including CRISPR–Cas systems and modular cloning in both conventional (*Saccharomyces cerevisiae*) and non-conventional (*Yarrowia lipolytica*, *Rhodotorula toruloides*, *Candida krusei*) yeasts. Additionally, genome-scale metabolic models and machine learning approaches have accelerated efforts to create a broad range of compounds that help reduce dependency on fossil fuels, mitigate climate change, and offer sustainable alternatives to petrochemical-derived counterparts. In this review, we highlight the cutting-edge genetic tools driving yeast metabolic engineering and then explore the diverse applications of yeast-based platforms for producing value-added products. Collectively, this review underscores the pivotal role of yeast biotechnology in efforts to build a sustainable bioeconomy.

Keywords: yeasts; genetic engineering; chemical production; microbial fermentation

Introduction

Yeasts have played a pivotal role in our history even before understanding their existence as they enabled the production of essential metabolites like ethanol, acetic acid, and lactic acid through natural fermentation processes (Maicas 2020, Voidarou et al. 2021). These native metabolites have been a mainstay in the food and beverage industries for centuries, contributing to products like bread, beer, and yogurt. However, with the rise of genetic engineering and sequencing technologies, yeast has evolved beyond this traditional food fermentation role (Jin et al. 2020). Specifically, modern tools now enable precise genome manipulation and the introduction of heterologous pathways thereby transforming yeast into a versatile microbial cell factory capable of producing high-value chemicals tailored to industrial needs (Lee et al. 2021, Koh et al. 2024).

At the recent forefront of this transformation are tools such as CRISPR–Cas9, which provides programmable genome editing, and auxiliary systems like CRISPR interference and CRISPR activation for tunable gene regulation (Deaner and Alper 2019, Bowman et al. 2020, Liu et al. 2022). Additionally, synthetic biology has advanced efforts to introduce modular cloning (MoClo) systems, synthetic promoters and terminators, create dynamic regulatory circuits, and facilitate the construction of efficient metabolic pathways. These advances, combined with systems biology approaches, have made yeasts a rather potent and tractable set of host organisms (Perrot et al. 2024, Yuan et al. 2024).

While *Saccharomyces cerevisiae* remains a cornerstone of yeast biotechnology due to its genetic tractability and industrial relevance/track record, there are many limitations that remain including substrate utilization profile and stress tolerance that have thus driven interest in non-conventional yeasts such as *Yarrowia lipolytica*, *Rhodotorula toruloides*, and *Candida krusei* (*Issatchenkia orientalis*), among others. These emerging yeast hosts exhibit unique metabolic traits, robust growth under stressful conditions, and versatile carbon source utilization (Blazeck et al. 2014, Wagner and Alper 2016, Koh et al. 2023). Despite these advances, many key challenges in the engineering of these hosts.

With these contexts in mind, this review explores the transformative impact of advanced genetic engineering tools on yeast biotechnology. First, we highlight how CRISPR systems, MoClo frameworks, and RNA-based regulatory strategies have expanded the capabilities of both traditional and non-conventional yeasts. Next, we examine trends in the production of biofuels, organic acids, terpenoids, and specialty chemicals, offering an overview of how cutting-edge innovations are shaping the future of yeast-based biomanufacturing. Finally, we look conclude with visions for the future of yeast host organisms to drive sustainable solutions for global industrial needs.

Advances in genetic engineering tools for yeast

A number of emerging genetic approaches are available for yeast hosts. Figure 1 demonstrates available engineering tools in yeast genetic engineering studies that are further described in this section.

CRISPR–Cas systems

The advent of CRISPR–Cas systems have revolutionized yeast metabolic engineering by enabling precise, efficient, and pro-

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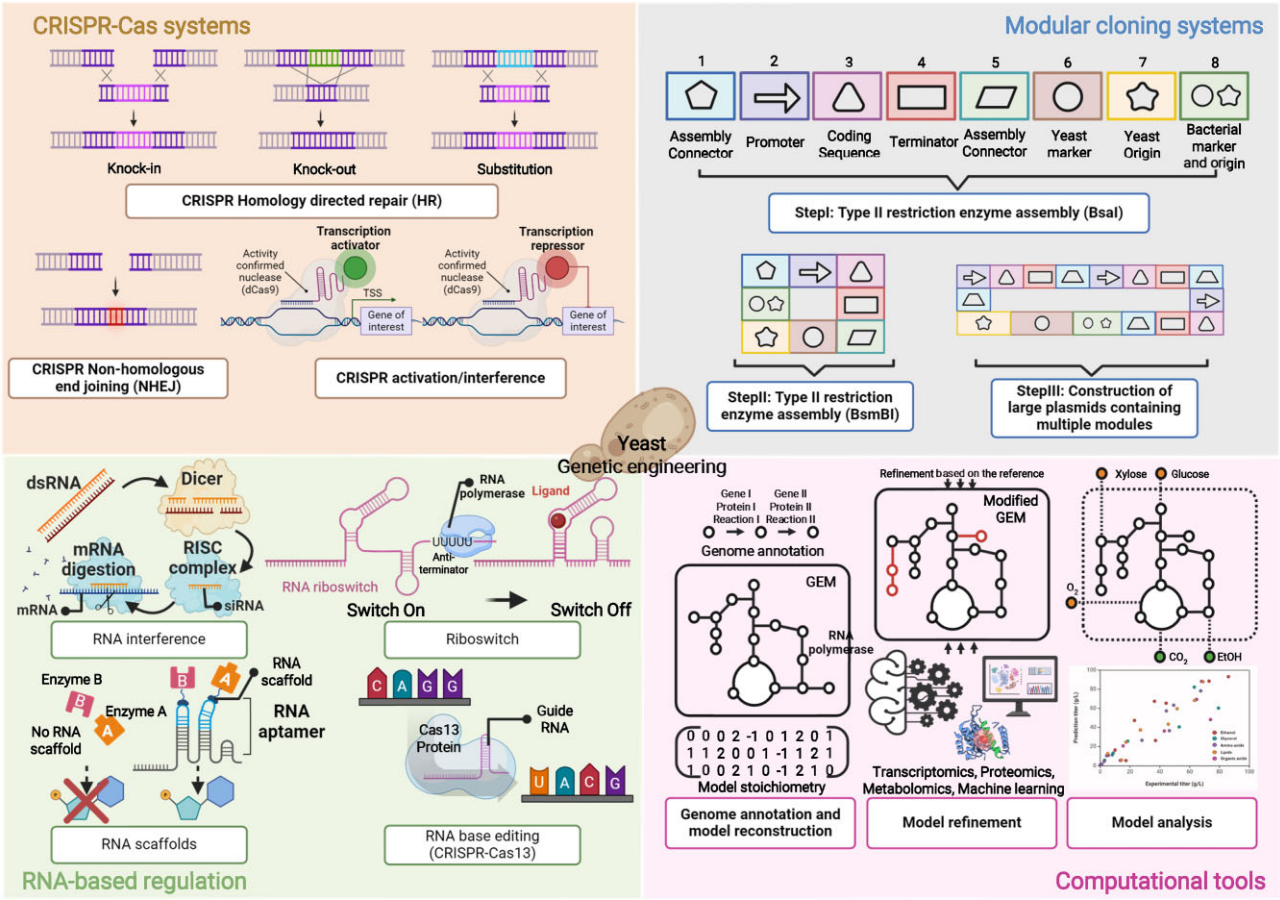

Figure 1. *Available engineering tools for yeast genetic engineering*. CRISPR–Cas and modular cloning systems have been extended beyond model yeasts to non-conventional yeasts, enabling more efficient construction of expression cassettes for genetic modifications. While RNA-based regulation has been predominantly applied to model yeasts, its implementation in non-conventional yeasts is expected to facilitate faster and more effective transcriptional regulation, driving advancements in yeast-based biotechnology. The integration of transcriptomics, metabolomics, and proteomics, coupled with machine learning to enhance genome-scale metabolic models, is anticipated to significantly improve the DBTL (Design, Build, Test, Learn) cycle in yeast genetic engineering (This figure was created with Biorender.com; https://BioRender.com/d99r330).

grammable genome editing as well as transcriptional regulation control systems (Stovicek et al. 2017). Among these, CRISPR–Cas9 has emerged as the most widely used system, consisting of the Cas9 protein, CRISPR RNA (crRNA), and trans-activating RNA (Li et al. 2023). In *S. cerevisiae*, where homologous recombination (HR) is the dominant mechanism of DNA repair, CRISPR–Cas9 can facilitate efficient genome editing including the insertion of large sequences of DNA with high precision (Jakočiūnas et al. 2015, Liu et al. 2019, Utomo et al. 2021, Meng et al. 2023). In contrast, non-conventional yeast such as *Y. lipolytica* rely predominantly on non-homologous end-joining (NHEJ) (Kretzschmar et al. 2013). This prevalence of NHEJ can result in unintended insertions or deletions, thus making knock-in efficiencies substantially lower in non-conventional yeasts when compared to *S. cerevisiae*. In addition to DNA editing, CRISPR systems have been adapted for precise RNA-level regulation. Jing et al. (2018) employed LshCas13a in *Schizosaccharomyces pombe*, achieving transcript knockdown efficiencies of up to 60%, but adaptation to *S. cerevisiae* revealed limitations in expression and activity. To address these challenges, a dCas13a system fused with hADAR2d enabled targeted RNA base editing in *S. cerevisiae*, achieving ~20% efficiency for endogenous transcripts.

While CRISPR tools for DNA editing are well-established for *S. cerevisiae*, extending these systems to non-conventional yeasts has faced significant challenges due to differences in DNA repair mechanisms, transformation efficiencies, and tool availability (Cai et al. 2019). The application of CRISPR–Cas9 in *Y. lipolytica* is limited by its reliance on NHEJ as the dominant DNA repair mechanism, but has been demonstrated in high-throughput (Schwartz et al. 2019, Lupish et al. 2022). Efforts to suppress NHEJ and enhance HR, such as deleting KU70/80 genes, have improved editing efficiencies, but often at the cost of cell growth and stability (Schwartz et al. 2016, Ploessl et al. 2022, Yuzbashev et al. 2023). Other efforts to improve editing efficiency include eliminating the intergenic sequence between tRNA and sgRNA to directly fuse the two components (Abdel-Mawgoud and Stephanopoulos 2020). Regardless of the approach, the efficiency of knock-in remains heavily dependent on target loci and the size of integration cassettes, making CRISPR-mediated genome editing in *Y. lipolytica* more labor-intensive and less efficient than in *S. cerevisiae*.

Genome editing in *R. toruloides* is constrained by low transformation efficiencies and the absence of episomal expression systems (Yu and Shi 2023). Consequently, Cas9 and sgRNA must be integrated chromosomally prior to editing and regulation efforts. Studies have successfully deleted multiple genes, including CAR2, URA3, CRTYB, however, due to limitations in the DNA repair mechanisms, knock-in approaches have not been extensively explored, and further research is needed to improve their

efficiency (Otoupal et al. 2019, Schultz et al. 2019). Finally, transformation efficiency is still low (≤1000 colonies/μg DNA) (Liu et al. 2017, Schultz et al. 2022) and thus remains a major limitation for genetic engineering in *R. toruloides*.

Despite sharing HR as the primary DSB repair mechanism with *S. cerevisiae*, genetic engineering in *C. krusei* has been limited by the lack of genetic tools. Recent breakthroughs include the development of a plasmid containing *S. cerevisiae-derived autonomously replicating sequence* s, enabling plasmid-based replication in *C. krusei* (Tran et al. 2019). This advance has facilitated the use of CRISPR–Cas9 to knock out the *ARD2* gene followed by successful integration of the xylose metabolism pathway into chromosomal intergenic regions (Cao et al. 2020). Therefore, CRISPR approaches in *C. krusei* are certainly emerging.

Beyond gene editing, the use of a catalytically inactive Cas9 (dCas9) has expanded CRISPR applications into transcriptional regulation. By fusing dCas9 to effector domains, gene expression can be modulated without inducing DNA breaks (Adli 2018). By fusing either activator or repressor domains to the dCas system, it is possible to enable either activation or repression, respectively (Zalatan et al. 2015). As an example of utility, Lian et al. (2017) developed a multifunctional CRISPR system for simultaneous activation, interference, and deletion, achieving a β-carotene-producing strain with threefold higher yields than the control in *S. cerevisiae*. This approach can also allow for the rapid prototyping of metabolic rewiring in *S. cerevisiae* (Deaner et al. 2018).

Additional CRISPR applications have expanded to include precise site-specific amino acid modifications through base editing and prime editing. Base editors, including cytosine base editors and adenine base editors, employ a catalytically impaired Cas9 (nCas9) fused to a deaminase, allowing targeted nucleotide conversions without inducing double-strand breaks (Kantor et al. 2020). In *S. cerevisiae*, this approach has been applied to introduce missense mutations into metabolic enzymes, facilitating enhanced catalytic efficiency and substrate specificity. For instance, Liu et al. (2021) leveraged Target-AID (nCas9 + cytidine deaminase) to engineer stress-tolerant yeast strains by targeting C-to-T substitutions in the SPT15 transcription factor. These engineered strains exhibited a 1.6-fold increase in biomass yield under ethanol stress and performed 14.3% faster fermentation under hyperosmotic conditions, demonstrating the utility of base editing in enhancing fermentation robustness. Separately, Cazier et al. (2023) developed a diversifying base editor by fusing dCas9 with a cytidine deaminase, enabling *in vivo* evolution of proteins. When applied to antibody engineering, this system facilitated a 100-fold enhancement in binding affinity, underscoring its potential in protein optimization. Prime editing, a more recent CRISPR-based tool, expands genome engineering capabilities by utilizing a Cas9 nickase fused to a reverse transcriptase, enabling precise codon substitutions, small insertions, and deletions without requiring double-strand breaks (Zhao et al. 2023). Although prime editing has historically exhibited lower efficiency compared to standard Cas9 nucleases (Kim et al. 2020), recent innovations have significantly improved its performance. The development of engineered prime editing guide RNAs (epegRNAs) with structured RNA motifs has enhanced pegRNA stability, increasing editing efficiencies by 3–4-fold (Nelson et al. 2022). Furthermore, OrthoRep-directed evolution of prime editors in yeast has resulted in PE_Y18, a highly optimized variant with up to 3.5-fold higher editing efficiency compared to PEmax (Weber et al. 2024).

Recent advances in CRISPR–Cas systems have profoundly transformed yeast metabolic engineering. Extending these tools to non-conventional yeasts still poses some significant challenges. Future research should focus on creating species-specific solutions to unlock the full potential of these non-conventional yeasts, thereby broadening their applicability in industrial biotechnology.

MoClo systems

MoClo system is a hierarchical DNA assembly technique designed to streamline the construction of multigene constructs (Weber et al. 2011). This method leverages Type IIS restriction enzymes, which generate unique overhangs by cutting outside their recognition sites, enabling seamless and directional assembly of multiple DNA fragments in a predetermined sequence without additional unwanted sequences. This system allows for rapid, flexible, and scalable assembly of complex genetic constructs (McGee et al. 2024). Recent advancements have extended these tools beyond simply conventional yeasts and have enabled rapid strain construction of non-conventional yeast as well. In *S. cerevisiae*, MoClo systems make use of pre-standardized DNA parts to allow researchers to mix-and-match various combinations of promoters, coding sequences, and regulatory elements to optimize gene expression and metabolic flux (Otto et al. 2021). Further expansions to the toolkit include panels of signal peptides and translational fusion partners to optimize heterologous protein secretion (O'Riordan et al. 2024). These systems can thus leverage the substantial advances made in the development of synthetic promoter and terminator elements for *S. cerevisiae* (Blazeck et al. 2012, Curran et al. 2013, 2015, Redden and Alper 2015, Presnell et al. 2024). Recent advancements in MoClo systems have significantly improved genomic integration capabilities, enabling the development of advanced multigene assembly techniques compatible with CRISPR–Cas9 systems (McCarty et al. 2019, Otto et al. 2021, Shaw et al. 2023). Particularly, Shaw et al. (2023) expanded these capabilities by identifying 10 novel intergenic loci within the *S. cerevisiae* genome for targeted integration. While this study provides a robust foundation for genomic modifications, these 10 loci do not necessarily represent the upper limit, as further research may uncover additional integration sites. Additionally, the introduction of 10 new yeast-selectable markers and the expansion of transcriptional unit (TU) capacity to 10 per construct have substantially enhanced the flexibility and scalability of the MoClo toolkit, facilitating the construction of more complex genetic circuits and the advancement of strain engineering.

For *Y. lipolytica*, several MoClo systems, including YaliBrick, EasyCloneYALI, and YALIcloneNHEJ, have been developed to facilitate efficient genetic engineering (Wong et al. 2017, Dahlin et al. 2021, Li et al. 2022). These toolkits enable the simultaneous assembly of multiple TUs such as promoters, coding sequences, terminators, and selection markers in a single reaction. They have been successfully employed in different studies to achieve distinct engineering objectives. For example, the EasyCloneYALI was used to construct an endogenous xylose metabolism pathway (ylXR: YALI0D07634g, ylXDH: YALI0E12463g, and ylXK: YALIF10923g), enabling the yeast to utilize xylose as the sole carbon source (Larroude et al. 2019). Additionally, YALIcloneNHEJ facilitated the establishment of synthetic pathways for terpenoid production, resulting in the production of (-)-α-bisabolol at a titer of 4.4 g/l (Li et al. 2022).

In *R. toruloides*, MoClo systems have bypassed the lack of episomal plasmids by adopting chromosomal integration strategies and standardized DNA parts optimized for the high GC-content of *R. toruloides* (Bonturi et al. 2022). This system includes a library of native promoters, terminators, resistance markers, and inser-

tional sequences. Targeting the KU70 locus, for example, facilitates homologous recombination and enabled a complete overexpressed carotenoid biosynthetic pathway that improved production by 41% compared with control strains (Bonturi et al. 2022).

MoClo efforts are lagging for *C. krusei*, but can likely borrow from recently identified genetic tools and approaches (Cao et al. 2020). Developing a MoClo system specifically for *C. krusei* would enable standardized genetic parts and streamlined pathway assembly, paving the way for more efficient and versatile applications of this acid-tolerant yeast in industrial biotechnology.

MoClo systems have the potential to transform the rate of progress in yeast systems (especially when combined with automated systems). While systems like MoClo have been well-established in *S. cerevisiae*, their adaptation to non-conventional yeasts represents a promising frontier. Expanding modular systems to yeasts such as *C. krusei* will further enhance their accessibility and scalability, ensuring their broader application in industrial biotechnology.

RNA-based regulation

RNA-level control (both with and without CRISPR-based systems) offers a precise, dynamic, and often reversible control of gene expression (Ranzani et al. 2024). These systems can be used to enable both fine-tuned optimization and more complex synthetic control systems (Dykstra et al. 2022). In *S. cerevisiae*, RNA tools such as RNA interference (RNAi), riboswitches, RNA scaffolds, and CRISPR–Cas13 have led to new control of cellular transcription and metabolism. RNAi, though absent in native *S. cerevisiae*, has been reconstituted using components from *Saccharomyces castellii* and human systems. This reconstitution includes Dicer (Dcr1) and Argonaute (Ago1), coupled with siRNA precursors transcribed as hairpins or double-stranded RNA (dsRNA) and has been used as an effective knockdown strategy suitable for improving tolerances and metabolite overproduction (Crook et al. 2014, Si et al. 2015). Expanding beyond single input regulation, Tian et al. (2024) developed RNAi-based Boolean logic gates using inducible and convergent promoter architectures to regulate siRNA precursor synthesis.

RNA in *S. cerevisiae* can also be used in a non-catalytic way to spatially organize biological components, such as enzymes, transcription factors, or RNA-binding proteins, within cells (Dykstra et al. 2022). Pothoulakis et al. (2022) introduced RNA origami scaffolds that fused sgRNAs with multiple functional RNA motifs (sgRNAOs) to recruit activators to target genes, thus enabling 2.7-times higher violacein production compared to the control. Yu and Marchisio (2024) likewise explored the use of RNA scaffold integrated together with type V CRISPR–Cas systems to yield up to 13-fold activation in synthetic gene circuits.

Despite all these successes in *S. cerevisiae*, applying RNA-based tools to non-conventional yeasts still remains a challenge. Species-specific differences in RNA stability, processing, and delivery hinder the adaptation of these tools, but can benefit from newer machine learning models (Hwang et al. 2024). Collectively, these RNA-based regulation tools can enable a versatile and scalable framework for enhancing yeast biomanufacturing and should be explored further in non-conventional yeasts.

Computational tools for metabolic engineering

Embracing a "systems metabolic engineering" approach has been able to complement these synthetic tools to help create a more predictable strain engineering approach (Presnell and Alper 2019, Choi and Lee 2023). In particular, fungal metabolic engineering has advanced significantly due to computational tools, particularly genome-scale metabolic models (GEMs) (Wang et al. 2017). Metabolic pathways simulations can help optimize production for a variety of industrial applications such as biofuel production, lipid synthesis, and high-value metabolite biosynthesis (Kundu et al. 2024). For *S. cerevisiae*, the iterative refinement of GEMs, such as the overall Yeast model (currently version 9) has improved prediction accuracy (Zhang et al. 2024). When combined together with strategies such as Flux Balance Analysis and Flux Scanning Based on Enforced Objective Flux, it is possible to rapidly design strains *in silico* strategies guiding the design of engineered strains (Zhang et al. 2024). For example, identification of targets to increase heme production led to the overexpression of HEM13, HEM14, and HEM2, and the deletion of SHM1, GCV1, and GCV2 enabling a production titer of 53.5 mg/l (a 70-fold increase in productivity) (Ishchuk et al. 2022).

For non-conventional yeasts, GEMs are becoming increasingly available and comprehensive. The development of GEMs for *Y. lipolytica* originated from homology-based reconstruction using *S. cerevisiae* models and have then become curated to reflect the strain's distinctive metabolic traits (esp. fatty acid catabolism and anabolism) (Loira et al. 2012, Kavšček et al. 2015). Xu et al. (2020) evaluated existing *Y. lipolytica* GEMs such as iMK735 and iYL_2.0 as a way to address gaps in the model leading to an improved GEM, iYL_2.0_corr that more accurately captured biomass reactions and pathway definitions. Additionally, an environmental version of MOMA (eMOMA) was applied to the iMK735 model to optimize lipid production in *Y. lipolytica* under nitrogen-limited conditions (M. Kim et al. 2019). The eMOMA-based predictions identified key genetic targets, including the overexpression of acetyl-CoA carboxylase (ACC1) and delta-9 stearoyl-CoA desaturase (SCD1), alongside a novel knockout target, YALI0F30745 g leading to an *in vivo* 45% increase in lipid accumulation over the wild-type.

In a similar fashion, GEMs have been applied to *R. toruloides* (rhto-GEM) by leveraging curated models of *S. cerevisiae* (Yeast 8.2.0) and *Y. lipolytica* (iYali 4.1.1) and incorporating experimental data and using the SLIMEr formalism (Tiukova et al. 2019). Building upon these types of approaches, Dinh et al. (2019) developed the iRhto1108 GEM based on Yeast 7.6 along with integrated transcriptomics data (especially in the nitrogen-limited conditions) to achieve a model with a MEMOTE accuracy score of 87%, a marked improvement over the prior models. Furthermore, Reķēna et al. (2023) developed condition-specific enzyme-constrained GEMs (ecRhtoGEMs) by integrating absolute proteome quantification and detailed physiological datasets under several carbon sources to more accurately predict lipid yields.

To date, only one GEM has been reported for *C. krusei*. Suthers et al. (2020) developed the genome-scale model iIsor850, incorporating 850 genes and 1826 reactions, based on *S. cerevisiae* GEMs (Yeast 7.6 and Yeast 8.3.4) and KBase data. With a MEMOTE score of 84%, the model was validated for succinic acid production, achieving an experimentally confirmed titer of 11.6 g/l under optimized conditions.

The development and use of GEMs supported by emerging computational techniques and integrated multi-omics data continues to expand the horizons of yeast metabolic engineering. By enabling precise metabolic predictions, these tools play a pivotal role in the strain design process. Clearly more efforts need to be focused on refining these models for non-conventional hosts as described above. When combined with the various synthetic tools

available, there is considerable promise for yeast strain engineering.

Trends in chemical production from yeasts

The tools of metabolic engineering and synthetic biology enable new control over metabolism. At the same time, microbial systems, in general, have a substantial breadth of achievable metabolic products (Lee et al. 2019). For yeasts in particular, a number of key classes of accessible models (Fig. 2) can help drive the future of fungal biotechnology. We highlight the broad classes of bio-based products derived from yeast here and present a summary of their biochemical production in Table 1.

Biofuels

Yeast-based biofuel production has long been a cornerstone of the sustainable energy field owing to renewable ethanol production (Kiran et al. 2014) and expanding to advanced fuels like butanol and isobutanol (Kamalesh et al. 2024). Bioethanol remains the most extensively studied and commercially utilized yeast-based biofuel (Beluhan et al. 2023). In particular, *S. cerevisiae* remains the primary organism of choice for bioethanol production. Efforts to further improve this innate capacity includes overexpressing genes such as PDC1 and ADH1 to more efficiency improve ethanol production, suppression of GPD1 and GPD2 to reduce glycerol byproduct formation, and fermentation optimization to maximize production (Hubmann Georg et al. 2011, Mohd Azhar et al. 2017, Papapetridis et al. 2017). Alternative carbon sources (including lignocellulose biomass) has been explored to reduce costs but require strain engineering to bypass the suppression of inhibitors such as furfural, acetic acid, and phenolics (Liu et al. 2019). To this end, adaptive laboratory evolution (ALE) and CRISPR-based genome editing have been applied to enhance yeast tolerance and identified mutants in transporter genes (HXT15, HXT11) and detoxification pathways (PAU, CUP1) (My et al. 2025). Finally, efforts to enable co-fermentation of glucose and xylose (the major constituent sugars in lignocellulose hydrolysate) have been developed by introducing sweet transporters from *Lipomyces starkeyi* (LST1_025 437) and *Arabidopsis thaliana* (AtSWEET7) (Kuanyshev et al. 2021) as well as through transporter protein engineering (Li et al. 2016).

Although the availability of metabolic engineering tools for *C. krusei* is currently limited, this yeast has emerged as a promising platform for bioethanol production due to its notable thermotolerance, acid resistance, and inhibitor resilience. *C. krusei* demonstrates superior growth and ethanol productivity under challenging industrial conditions, including elevated temperatures, low pH, and the presence of toxic inhibitors commonly found in lignocellulosic hydrolysates (Zwirzitz et al. 2021). For example, *C. krusei* achieved ethanol production of 41.4 g/l with a productivity of 1.5 g/l/h at 42°C and pH 4.2, even in the presence of 4 g/l acetic acid where *S. cerevisiae* exhibited significantly reduced performance (Seong et al. 2017). Furthermore, in mixed cultures with *S. cerevisiae*, *C. krusei* has shown rapid ethanol production at temperatures as high as 42°C, surpassing *C. krusei* in both production rate and biomass accumulation (Gallardo et al. 2011). Genomic and transcriptomic analyses have revealed that the enhanced resilience of *C. krusei* can be attributed to the differential expression of genes involved in ergosterol biosynthesis, trehalose metabolism, and stress response pathways (Miao et al. 2018). These adaptations enable the yeast to maintain membrane integrity and protein homeostasis under stressful environments, thereby supporting its superior ethanol production capabilities. These traits position *C. krusei* as a robust alternative to traditional bioethanol-producing strains, particularly for industrial processes involving harsh conditions.

Similar efforts in other non-conventional yeasts such as *Y. lipolytica* and *R. toruloides* have expanded the potential for yeast-based biofuel production. *Yarrowia lipolytica* is particularly suited for biodiesel production due to its ability to accumulate lipids up to 90% of its dry cell weight (Blazeck et al. 2014, Yook et al. 2019). Metabolic engineering efforts have enhanced triacylglycerol (TAG) biosynthesis by overexpressing key enzymes like acetyl-CoA carboxylase (ACC) and diacylglycerol acyltransferase (DGA), while suppressing competing pathways through deletions such as pex10 (peroxisomal biogenesis factor 10) and mfe1 (peroxisomal hydroxyacyl-CoA dehydrogenase) (Tai and Stephanopoulos 2013, Liu et al. 2015, Park and Nicaud 2020). ALE and CRISPR–Cas9 have further improved *Y. lipolytica*'s ability to utilize non-conventional carbon sources, such as xylose (Rodriguez et al. 2016, Li and Alper 2020, Yook et al. 2020). Recent reports suggest that amplifying oxidoreductase pathway genes and introducing mutations in GTPase-activating protein (YALI0B12100p) significantly enhance biodiesel production from lignocellulosic hydrolysates (Yook et al. 2025). Moreover, strategies to enhance the production of fatty acid ethyl esters (FAEEs) and free fatty acids (FFAs) (including overexpressing wax ester synthase genes and deleting FAA1) have achieved extracellular FFA levels of up to 4.3 g/l (Gao et al. 2018, Salvador López et al. 2023).

R. toruloides has attracted attention for its innate ability to produce over 50% dry cell weight as lipids without genetic modifications (Qi et al. 2020, Wen et al. 2020). Moreover, this organism can natively utilize xylose making it a promising candidate for converting lignocellulosic sugars (Lee et al. 2021, Koh et al. 2024, Wang et al. 2024). Genetic engineering efforts such as overexpressing native ACC1 and DGA1, have increased lipid synthesis by 74.6% (14.2 to 24.8 g/l) in batch mode and 348% (18.0 to 62.8 g/l) in fed-batch mode (Zhang et al. 2016). Additional overexpression of SCD1 further boosted lipid production to 89.4 g/l in fed-batch fermentations. In a subsequent study, the strain achieved lipid synthesis of 8.8 g/l of lipid using oil cane hydrolysate as the feedstock in a 75-l batch fermentation (Deshavath et al. 2024). Despite advantages of this strain on a metabolic level, further enhancements are necessary to improve tolerance to high substrate concentrations, osmotic stress, and inhibitory compounds (Kamal et al. 2024, Sunder et al. 2024).

Beyond ethanol and biodiesel, significant advances have been made to produce advanced biofuels such as butanol, isobutanol, and higher alcohols that have higher energy densities (Connor and Liao 2009). Pathway complementation is typically necessary for engineering these pathways into yeast. For instance, isobutanol production has been achieved in *S. cerevisiae* by introducing the ketoisovalerate decarboxylase (KIVD) pathway from *Lactococcus lactis* together with endogenous enzymes Ilv2p, Ilv3p, and Ilv5p in the cytosol, isobutanol production of 151 mg/l was observed in microaerobic conditions (Lee et al. 2012). Building on these foundational studies, recent advancements have focused on improving isobutanol production through systems-level metabolic engineering. A notable example involves the overexpression of the transcription factor Znf1, which regulates genes in the pentose phosphate pathway and isobutanol biosynthesis (Songdech et al. 2024), and led to 14.8 g/l of isobutanol production from xylose in a 5-l bioreactor.

Yeast-based biofuels represent a pivotal solution for transitioning to sustainable energy systems. As the global energy landscape evolves and moves to more energy dense molecules beyond

Table 1. Overview of biochemical production by yeast strains.

Product	Organism[1]	Carbon source[2]	Genotype	Titer (g/l)[3]	Productivity (g/l∗h)[3]	Reference
Biofuel						
Ethanol	SC	Glucose with acetic acid (M)	ura3-52 MAL2-8c SUC2 can1::cas9-natNT2 gpd1::gpsA gpd2::eutE ald6Δ	82.8	1.725	Papapetridis et al. (2017)
Ethanol	SC	Glucose (M)	GPD1p::TEFmut7 GPD2p::TEFmut7	24.23	1.13	Hubmann et al. (2011)
Ethanol	CK	Glucose (C)	isolate 195B	40	3.33	Gallardo et al. (2011)
Ethanol	CK	Glucose (C)	MTY1	41.4	1.5	Seong et al. (2017)
Ethanol	CK	Wheat straw hydrolysate (C)	KJ27-7	10.3	0.43	Zwirzitz et al. (2021)
Lipid	YL	Glucose (M)	pex10Δ mfe1Δ leucine + uracil + DGA1	25.3	0.21	Blazeck et al. (2014)
Lipid	YL	Glucose (M)	mga2-G643R pex10Δ DGA1	25	0.145	Liu et al. (2015)
Lipid	YL	Glucose (C)	ACC1::hp4d DGA1::TEFin	28.5	0.143	Tai *et al.* (2013)
Lipid	YL	Glucose and Xylose (M)	XDH::UAS1B8-TEF XKS::UAS1B8-TEF	G: 0.75 X: 0.2	NA	Rodriguez et al. (2016)
Lipid	YL	Xylose (M)	Xyl3A∗ ylXK DGA1	12.01	0.097	Yook et al. (2020)
Lipid	YL	Xylose (M)	ssXR ssXDH ssXK	2.5	0.028	Yook et al. (2025)
Lipid	RT	Glucose and Xylose (C)	ACC1::GAPDH DGA1::ACL	G: 16.4 X: 9.5	G: 0.0756 X: 0.0247	Zhang et al. (2016)
Lipid	RT	Oilcane hydrolysate (C)	ACC1::GAPDH DGA1::ACL	8.8	0.0611	Deshavath et al. (2024)
Organic acid						
Lactic acid	SC	Glucose (M)	LDH JEN1 ADY2 PDC1Δ ADH1Δ PHO13Δ ALD6Δ GPD1Δ	121.5	1.69	Zhu et al. (2022)
Lactic acid	SC	Xylose and Synthetic hydrolysates (C)	LDH ERF2Δ CYB2Δ GPD1Δ PHO13Δ ALD6Δ CDC19	X: 93 S: 30	X: 0.78 S: 0.21	Choi et al. (2024)
Lactic acid	CK	Mixed sugar (glucose + xylose) (C)	SD108 PDCΔ GPDΔ LDH XR XDH XK	67.1	0.59	Lee et al. (2024)
Succinic acid	CK	Glucose (C)	GPD1p GPD2p MAE1 pdcΔ gpdΔ g3473Δ ndeΔ	109.5	0.54	Tran et al. (2023)
Succinic acid	YL	Glucose (C)	*Sc*PCK *Ec*Fum YlMdh1 YlMdh2	111.9	1.79	Cui et al. (2023)
Succinic acid	YL	Crude glycerol (C)	SDH5Δ	209.7	1.45	Li et al. (2018)
Citric acid	YL	Glucose (M)	YHM2p::hp4d YlAMPDp::hp4d	97.1	0.93	Yuzbasheva et al. (2019)
Citric acid	YL	Glucose (M)	PYC1p::hp4d	111.1	0.46	Fu et al. (2016)
Citric acid	YL	Con stover hydrolysate (C)	ICL1p YHM2p AMPDp DGA1Δ	83.6	0.50	Lu et al. (2025)
3-HP	SC	Glucose (M)	PYC1 PYC2 *Bc*BAPAT *Ec*YDFG *Tc*PAND	13.7	0.17	Borodina et al. (2015)
3-HP	SC	Glucose (M)	PYC MdlC MmsB	18.1	0.17	Tong et al. (2021)
3-HP	YL	Glucose (C)	tMCRC tMCRN *sm*GAPN ACC1 ALD6 ACS^{L641P} MLSΔ CIT2Δ HPDHΔ MMSDHΔ	16.23	0.068	Liu et al. (2023)
3-HP	RT	Glucose (C)	MCR g2945 ALD6Δ	45.4	0.44	Liu et al. (2023)

Table 1. Continued

Product	Organism[1]	Carbon source[2]	Genotype	Titer (g/l)[3]	Productivity (g/l∗h)[3]	Reference
Terpenes and isoprenoids						
α-Amyrin	SC	Glucose (M)	tHMG1 *Md*OSC1$^{N11T/P250H/P373A}$ DGA1	1.107	0.008	Yu et al. (2020)
Texadiene	SC	Glucose (C)	TASY–ERG20^{F96C}	0.236	0.0011	Nowrouzi et al. (2020)
Squalene	SC	Glucose (C)	ERG10 ERG13 tHMG1, ERG12 ERG8 ERG19 IDI1 ERG20 ERG9	21.1	0.151	Zhu et al. (2021)
Limonene	SC	Glucose (C)	*Sc*CK *At*IPK *Cl*LS	20.2 (mg/l)	0.168 (mg/l∗h)	Ma et al. (2023)
α-Farnesene	YL	Glucose (C)	ERG10(AtoB) HMGS HMGR α-FS	25.55	0.089	Liu et al. (2019)
α-humulene	YL	Glucose (C)	ERG10 ERG13 NADH-HMG ERG12 ERG8 ERG19 IDI ERG20 ACHS2	3.2	0.019	Guo et al. (2021)
D-Limonene	YL	Glucose (C)	tHMG1 ERG12 IDI ERG20^{N119W} NDPS1 D–LS	69.3 (mg/l)	0.5775 (mg/l∗h)	Park et al. (2024)
Lycopene	YL	Glucose (C)	IDI CrtE CrtB CrtI IPK CK ERG20	4.2	0.016	Luo et al. (2020)
Limonene	RT	Glucose (C)	*Ef*MvaE *Ef*MvaS *Mm*MK *Slt*NPPS1 *Clt*LS1	0.394	0.0033	Liu et al. (2021)
1,8–cineole α–bisabolene	RT	Corn stover hydrolysate (C)	1,8: HYP3 GgFPS(N144W) DaHMGR McMK ScPMK α: BIS SpHMGR McMK SpPMK	1,8: 1.4 α: 2.6	1,8: 0.008 α: 0.015	Kirby et al. (2021)
Other high-value products						
Lovastatin	SC	Glucose (C)	lovB lovC lovA lovG	35 (mg/l)	0.7(mg/l∗h)	Xu et al. (2013)
m-cresol	SC	Glucose (C)	*Pp*MSAS *Nan*pgA patG	0.589	0.0042	Hitschler *et al.* (2019)
TAL	SC	Glucose (C)	ACC1^{S686A,S659A,S1157A} RppA *Gh*2PS Adr1c^{S230A} Oaf1c Pip2c,	0.16	NA	Lin et al. (2023)
TAL	SC	Xylose + acetate (C)	XR XDH XK *Gh*2PS	23.91	0.29	Sun et al. (2021)
TAL	YL	Glucose (C)	ACC1 ACS1 ALD5 PDC2 PEX10 *Gh*2PS	35.9	0.21	Markham et al. (2018)
TAL	RT	Glucose and oilcane juice (C)	*Gh*2PS ACL1 ACC1	G: 28 O: 23	G: 0.23 O: 0.19	Cao et al. (2022)
TAL	RT	Sorghum hydrolysate (C)	*Gh*2PS	3.9	0.045	Otoupal et al. (2022)
Apigenin, Isovitexin	YL	Glucose (M)	ARO4 PEX10 *Se*SAM8 *Nt*4CL *Hs*PKS1 *Pc*FS1 GtUF6CGT1	A: 0.168 I: 0.119	NA	Marsan et al. (2024)
Resveratrol	SC	Glucose and Ethanol (M)	*Ha*TAL At4CL1 *Vv*STS ARO^{4K229L} ARO7^{G141S} ACC1$^{S659A/S1157A}$	G: 0.416 E: 0.531	G: 0.042 E: 0.053	Li et al. (2015)
Resveratrol	SC	Glucose (M)	*Rt*PAL *Pc*4CL *Vv*STS AtC4H AtCPR1 ARO4^{K229L} ARO7^{G141S} ACC1$^{S659A/S1157A}$	4.1	0.034	Meng et al. (2023)
Resveratrol	YL	Glucose (M)	*Fj*TAL *Pc*4CL1 *Vv*STS DGA1 Δ	22.5	0.16	Liu et al. (2022)
Resveratrol	RT	Glucose (C)	*At*4CL *Vl*STS AtC4H *At*ATR2 *Rt*CYB5 ARO4^{K227L} ARO7^{G153S}	0.125	0.0013	Zhang et al. (2022)

[1]: SC: *Saccharomyces cerevisiae*, CK: *Candida krusei*, YL: *Yarrowia lipolytica*, RT: *Rhodotorula toruloides*
[2]: C: Complex medium M: Minimal medium
[3]: According to Yook and Alper (2025), yield calculation from primary carbon sources is challenging when complex media was used; thus, strain performance is compared using only titer and productivity in this table.

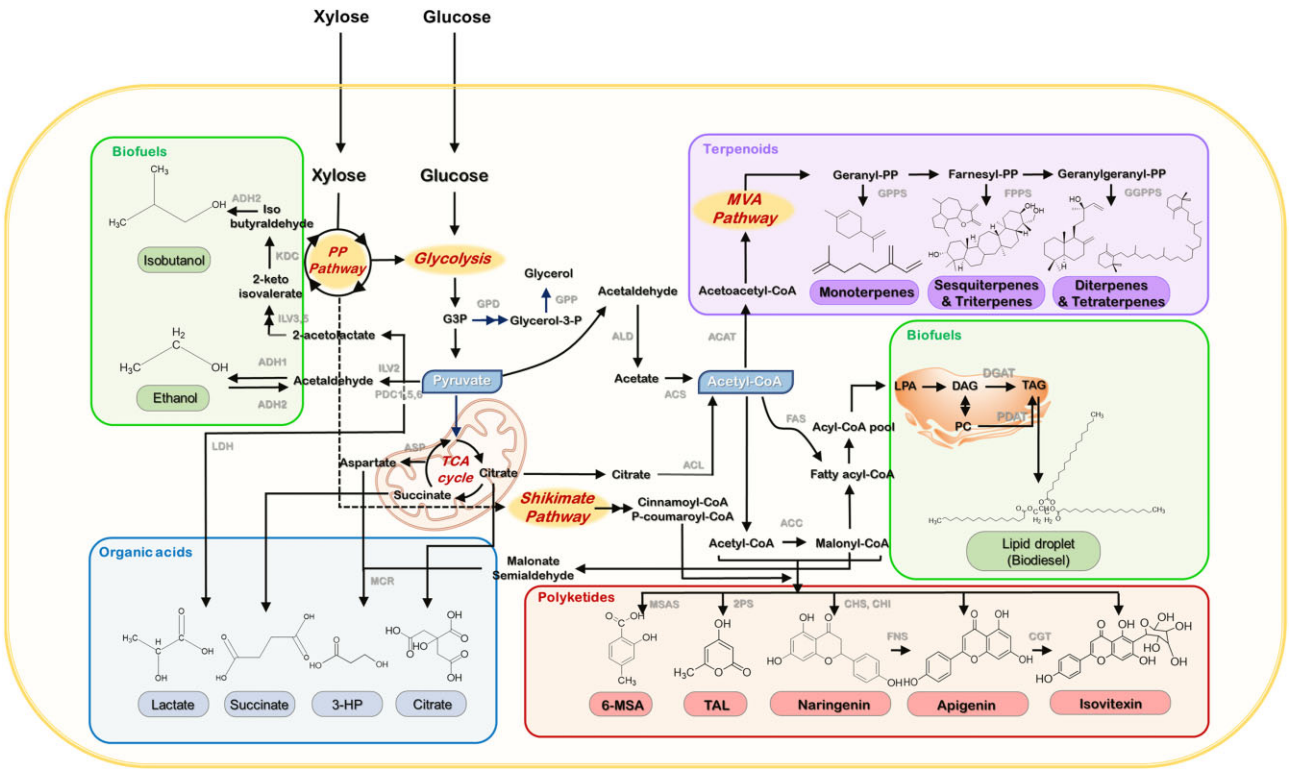

Figure 2. *Summary of metabolic pathways to produce biofuels, organic acids, terpenoids, and other value-added products in yeast species (S. cerevisiae, Y. lipolytica, R. toruloides, C. krusei).* Enzymes: ADH, alchol dehydrogenase; KDC, 2-keto decarboxylase; ILV3, dihydroxyacid dehydratase; ILV5, ketol-acid reductoisomerase; ILV2, acetolactate synthase; PDC1,5,6, pyruvate decarboxylases; LDH, lactate dehydrogenase, ASP, aspartic protease; MCR, Malonyl-CoA reductase; GPD, glycerol-3-phosphate dehydrogenase; GPP, glycerol-3-phosphatase; GPPS, geranyl diphosphate synthase; FPPS, farnesyl pyrophosphate synthase; GGPPS, geranylgeranyl pyrophosphate synthase; ACAT, acetyl-CoA C-acetyltransferase; ACS, acetyl-CoA synthetase; ACL, ATP-citrate lyase; FAS, fatty acid synthase; DGAT, diacylglycerol o-acyltransferase; PDAT, phospholipid:diacylglycerol acyltransferase; ACC, acetyl-CoA carboxylase; MSAS, 6-methylsalicylic acid synthase; 2PS, 2-pyrone synthase; CHS, chalcone synthase; CHI, chalcone isomerase; FNS, flavone synthase; CGT, C-Glucosyltransferase.

ethanol, the role of yeast as a versatile and scalable biofactory should remain.

Organic acids

Fungal production of organic acids has been a mainstay as well as a microbial biomanufacturing platform (Tönjes et al. 2024). Indeed organic acids, including lactic acid, succinic acid, citric acid, and 3-hydroxypropionic acid (3-HP), serve as key building blocks for a variety of applications, including bio-based polymers, food additives, pharmaceuticals, and chemicals (Chen and Nielsen 2016, Mazzoli 2021). Yeast species can uniquely tolerate acidic environments which is critical when contemplating production of a product with low pKa such as organic acids (Fletcher et al. 2017). While *S. cerevisiae* has been extensively engineered for organic acid biosynthesis, its limited TCA cycle flux poses challenges for producing TCA (Hayakawa et al. 2018). In contrast, non-conventional yeasts such as *Y. lipolytica* and *C. krusei* possess much stronger TCA cycle flux and exhibit much higher innate acid tolerances (Cavallo et al. 2017, Suthers and Maranas 2022, Pyne et al. 2023). Not surprising, recent strain and pathway engineering have led to substantial improvements in the titer, yield, and productivity of organic acids using yeast-based systems.

For lactic acid production, *S. cerevisiae* has been engineered to overcome its natural pathway inefficiencies to produce appreciable titers. As an example, heterologous expression of L-lactate dehydrogenase (LDH) genes from *Lactobacillus* species together with strategies to minimize competing pathways, such as Δ*ald6* (ethanol production) and Δ*gpd1* (glycerol production) enabled aerobic lactic acid production of up to 121.5 g/l with a yield of 0.81 g lactate/g glucose (Zhu et al. 2022). Flux models have likewise been used to enhance production from xylose through the overexpression of genes such as CDC19, LDH, and deletions of PHO13, ALD6, GPD1, and CYB2 (Choi et al. 2024). This strategy led to 93 g/l lactic acid with a yield of 0.84 g/g xylose from synthetic lignocellulosic hydrolysates. Efforts in non-conventional yeasts such as *C. krusei* have been explored owing to robust growth under low pH (up to 2.0) (Hisamatsu et al. 2006). A recent study demonstrated 67 g/l of D-lactic acid with a yield of 0.56 g/g from synthetic lignocellulosic hydrolysates using a self-buffering fermentation process to eliminate external pH control (Lee et al. 2024).

Succinic acid, a pivotal platform chemical for bio-based materials, has also been successfully produced using engineered yeasts. *S. cerevisiae* was initially explored for succinic acid production through the introduction of reductive, oxidative, and glyoxylate pathways, but redox imbalance—mainly the shortage of NADH due to ethanol production—limited production to under 10 g/l (Cheng et al. 2013, Li et al. 2021). In contrast, *Y. lipolytica* and *C. krusei* have been more suitable hosts. When *C. krusei* was optimized by integrating the reductive TCA pathway, introducing a dicarboxylic acid transporter (SpMAE1), and deleting ethanol and glycerol synthesis pathways to enhance NADH balance, it achieved 109.5 g/l succinic acid with a yield of 0.65 g/g from glucose and glycerol co-substrates (Tran et al. 2023). In *Y. lipolytica*, engineering efforts

included disrupting succinate dehydrogenase and introducing fumarate reductase (TbFrd) and malate dehydrogenase (YlMdh1), along with adaptive laboratory evolution to improve NADH availability enabled 111.9 g/l of succinic acid production with a yield of 0.79 g/g glucose and a productivity of 1.79 g/l/h in pilot-scale fermentation (Cui et al. 2023). Additionally, inactivating succinate dehydrogenase subunit 5 has been proven to be an efficient strategy for succinate production in Y. lipolytica, achieving a titer of 209.7 g/l with a productivity of 1.45 g/l/h (Li et al. 2018).

Citric acid production, historically dominated by a different fungal system, *Aspergillus niger*, has seen increasing contributions from alternative yeast systems, particularly *Y. lipolytica* (Cavallo et al. 2017), especially under nitrogen-limited conditions (Börekçi et al. 2021). Genetic modifications, such as overexpressing pyruvate carboxylase (PYC) and isocitrate lyase (ICL), and deleting ATP citrate lyase (ACL) to prevent citrate loss have yielded citric acid titers of 70–110 g/l with yields of 0.64–0.76 g/g from glucose (Fu et al. 2016, Yuzbasheva et al. 2019). In a more recent example, *Y. lipolytica* engineered to overexpress TCA cycle-related genes (AMPD, YHM2, ICL) and reduce lipid by-product formation (deletions of DGA1, DGA2, ACL) achieved a titer of 83.6 g/l citric acid with a yield of 0.44 g/g from undetoxified corn stover hydrolysates (Lu et al. 2025).

A variety of other organic acid products have been explored in yeasts. 3-hydroxypropionic acid (3-HP) is of strong interest as a versatile intermediate for bio-based plastics and chemicals (Ji et al. 2018). In *S. cerevisiae*, a novel oxaloacetate pathway was developed by overexpressing pyruvate carboxylase and other key enzymes to achieve 18.1 g/l in a 5-l bioreactor (Borodina et al. 2015). A synthetic β-alanine pathway was engineered, enabling a titer of 13.7 g/l with a yield of 0.14 C-mol/C-mol glucose in fed-batch fermentation (Tong et al. 2021). *Yarrowia lipolyty (IUP)) (Cica* has also been explored for 3-HP biosynthesis through the malonyl-CoA pathway, with genetic modifications such as overexpressing malonyl-CoA reductase and knocking out degradation-related genes, achieving a titer of 16.23 g/l in fed-batch fermentation (Liu et al. 2023). Moreover, *R. toruloides* demonstrated its potential by leveraging its high malonyl-CoA flux for 3-HP production from lignocellulosic hydrolysate, producing 45.4 g/l in fed-batch fermentation (Liu et al. 2023).

In summary, yeast-based systems have emerged as versatile and efficient platforms for the production of organic acids. Each yeast species offers unique advantages tailored to specific organic acids and thus pave the way for sustainable biomanufacturing of these products.

Terpenes and isoprenoids

The production of terpenes and isoprenoids using engineered yeasts has gained momentum due to their industrial applications in biofuels, pharmaceuticals, and fragrances (Zhang et al. 2017). Production of these molecules has required the integration of metabolic and protein engineering, organelle compartmentalization, and the development of synthetic pathways [such as the isopentenol utilization pathway (IUP)] (Clomburg et al. 2019, Li et al. 2022). In *S. cerevisiae*, the mevalonate (MVA) pathway is central for native generation of the C5 precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Rinaldi et al. 2022). Productivity increases were realized by overexpressing rate-limiting enzymes such as truncated HMG-CoA reductase (tHMG1) and integrating heterologous pathways like IUP (Chatzivasileiou et al. 2019, Yu et al. 2020). The IUP helped bypass the complexity of the native MVA pathway and resulted in nearly 147-fold increase in IPP/DMAPP pools and a 374-fold increase in monoterpene precursors such as geranyl pyrophosphate (GPP), enabling the production of limonene at titers of 20.2 mg/l (Ma et al. 2023). Further advances have completely replaced the MVA pathway to improve squalene accumulation by over 150% (26.02 mg/g/OD600). Moreover, limonene and β-carotene production reached 3.5 mg/g/OD600 and 2.0 mg/g/OD600, respectively, representing increases of 850-fold and 18-fold compared to strains that co-utilized the MVA and IU pathways (Li et al. 2024). Compartmentalization strategies (whether into the mitochondria, peroxisome, or endoplasmic reticulum) can further enhance production as seen with increased geraniol production to achieve squalene production of 21.1 g/l (Zhu et al. 2021). Additionally, protein engineering approaches in these hosts have improved enzyme specificity and activity 22-fold reaching 129 mg/l of taxadiene in flask fermentations (Nowrouzi et al. 2020).

In *Y. lipolytica*, native high acetyl-CoA flux has been exploited for terpenoid production and demonstrates its potential as a scalable platform. Engineering the MVA pathway and overexpressing key enzymes like ATP-citrate lyase have increased acetyl-CoA availability leading to the production of α-farnesene at 25.6 g/l and β-carotene at 39.5 g/l (Liu et al. 2019, Arnesen and Borodina 2022). Compartmentalization strategies, such as peroxisomal targeting, have significantly improved yields, achieving 3.2 g/l of α-humulene and 69.3 mg/l of d–limonene production, respectively (Guo et al. 2021, Park et al. 2024). Furthermore, the integration of the IUP has enhanced precursor availability, resulting in a lycopene titer of 4.2 g/l (Luo et al. 2020).

Emerging as a robust host, *R. toruloides* possesses a naturally high MVA pathway flux for terpenoid production. As such, the introduction of heterologous terpene synthases and pathway engineering has enabled the production of various terpenoids in this host. For instance, *R. toruloides* achieved limonene titers of 393.5 mg/l using a neryl pyrophosphate-based pathway, and 1,8-cineole production reached 1.4 g/l through the optimization of GPP synthase and upstream pathway enzymes (Kirby et al. 2021, Liu et al. 2021). The production of α-bisabolene at 2.6 g/l from lignocellulosic hydrolysates further highlights its industrial relevance (Kirby et al. 2021).

Yeast hosts are attractive choices for the production of terpenoid products. The integration of novel synthetic pathways, targeted organelle compartmentalization, and substrate-specific optimizations has led to dramatic improves in titers, yields, and overall productivity. Further engineering efforts can unlock yeasts' potential for sustainable, industrial-scale terpenoid production.

Other high-value chemicals

Beyond the compounds already discussed, yeasts have emerged as promising platforms for the production of other high-value chemicals, including polyketides, biopolymers, and specialty chemicals (Chattopadhyay et al. 2006, Jakočiūnas et al. 2020). Polyketides, a diverse group of secondary metabolites with pharmaceutical relevance (e.g. antibiotics, anticancer agents), are a major target for yeast-based production chemicals (Liu and Li 2022). These compounds are most commonly synthesized by polyketide synthases (PKSs) that catalyze the iterative condensation of malonyl-CoA and acetyl-CoA precursors (Zhou et al. 2023, Xiang et al. 2024). Initial efforts have focused on using *S. cerevisiae* owing to well-established genetic tools. In this regard, overexpressing acetyl-CoA carboxylase (ACC1) to increase malonyl-CoA availability, a critical precursor for polyketide biosynthesis along with heterologous expression for 6-methylsalicylic acid (6-MSA) production led

to 2 g/l production (Hitschler and Boles 2019). Similarly, heterologous expression of modular PKSs enabled the synthesis of complex polyketides like lovastatin precursors with yields exceeding 20 mg/l in optimized strains (Xu et al. 2013). A recent study compartmentalized ACC1 and type III polyketide synthases within the peroxisome achieving 46% increase in triacetic acid lactone (TAL) production to reach titers of 0.14 g/l (Lin et al. 2023). Another study demonstrated that cofeeding xylose and acetate reduced the Crabtree effect in *S. cerevisiae* and maximized acetyl-CoA production, increasing TAL production in a bioreactor to 23.91 g/l (Sun et al. 2021).

Oleaginous yeasts like *Y. lipolytica* and *R. toruloides* have a high potential for this class of molecules owing to superior capacity for acetyl-CoA and malonyl-CoA precursor accumulation. Markham et al. (2018) developed a pyruvate bypass pathway and enhanced β-oxidation by overexpressing PEX10 to achieve high TAL production of 35.9 g/l using glucose as the substrate. Further downstream engineering of this host led to the production of naringenin, a precursor for flavonoids, reached 898 mg/l followed by a modular bioconversion system to enable synthesis of apigenin and its glucoside derivative isovitexin at titers of 168 mg/l and 119 mg/l, respectively (Marsan et al. 2024). In a similar fashion, the introduction of 2-pyrone synthase (2-PS) enabled TAL production at 2.5 g/l in *R. toruloides* from synthetic media and 3.9 g/l using one-pot processed sorghum hydrolysates (Otoupal et al. 2022). Furthermore, metabolic engineering of acetyl-CoA pathways—such as overexpressing ACL1 and ACC1—has elevated TAL titers to 28 g/l in fed-batch fermentation with acetate supplementation, demonstrating a highly efficient conversion from low-cost substrates (Cao et al. 2022).

Specialty chemicals such as stilbenoids and benzylisoquinoline alkaloids (BIAs) are increasingly being targeted for yeast-based production due to their pharmaceutical and nutraceutical significance (Dai et al. 2015). For instance, resveratrol has been successfully produced in *S. cerevisiae* through the introduction of biosynthetic enzymes such as stilbene synthase (STS) and 4-coumaroyl-CoA ligase (4CL). By employing optimized metabolic pathways, including feedback-insensitive variants of $ARO4^{K229\,L}$ and $ARO7^{G141S}$ and enhanced acetyl-CoA flux, researchers have achieved titers of 531 mg/l in fed-batch fermentation (Li et al. 2015). A recent study combined phenylalanine and tyrosine pathways by introducing a bi-functional phenylalanine/tyrosine ammonia lyase from *R. toruloides*, achieving 4.1 g/l resveratrol production in *S. cerevisiae* (Meng et al. 2023). Likewise, *S. cerevisiae* has been extensively engineered to reconstitute complex biosynthetic pathways, achieving titers of 425.2 mg/l of (S)-reticuline using optimized enzyme combinations and multi-copy gene integration. These engineering efforts have extended to protoberberines and benzophenanthridine alkaloids, where the introduction of compartmentalized enzymes, such as berberine bridge enzyme, has significantly increased titers of intermediates like (S)-scoulerine by over 200%, reaching 113 mg/l. These advancements underscore *S. cerevisiae*'s versatility and scalability for producing high-value BIAs.

In *Y. lipolytica*, its oleaginous nature and high acetyl-CoA flux make it a promising host for stilbenoid production. Through multi-copy integration of biosynthetic pathways and precursor flux optimization, *Y. lipolytica* can produce upwards of 22.5 g/l of resveratrol in fed-batch fermentation (Liu et al. 2022). Similarly, *R. toruloides* has demonstrated potential for sustainable stilbenoid production achieving 125.2 mg/l of resveratrol in flask fermentation (Zhang et al. 2022). While *Y. lipolytica* and *R. toruloides* have not yet been used widely for BIA production, high upstream flux makes these strains promising candidates for future BIA biosynthesis. Collectively, these advancements highlight the complementary strengths of these yeast systems, paving the way for scalable and sustainable biosynthesis of specialty chemicals.

Concluding remarks

Yeast has moved far beyond basic genetic tools and ethanol production. Advances in genetic engineering tools, such as CRISPR–Cas systems, MoClo, and RNA-based regulation, have led to newfound control of metabolic pathways. These innovations, when combined with computational approaches, have expanded the metabolic landscape of traditional yeast models like *S. cerevisiae* and emerging non-conventional yeasts such as *Y. lipolytica*, *R. toruloides*, and *C. krusei* for industrial applications (Patra et al. 2021, Koh et al. 2024).

Despite these breakthroughs, challenges remain in addressing metabolic burden, product toxicity, and the limitations of genetic tools for non-conventional yeasts (Mao et al. 2024). Computational modeling and machine learning offer promising solutions, but their full potential has yet to be realized in these hosts, especially when datasets can be limited (Lu et al. 2022). Additionally, achieving economic scalability remains critical, emphasizing the need for cost-effective feedstocks, efficient downstream processes, and strong industry-academia collaboration (Makepa and Chihobo 2024). Looking ahead, yeast-based biomanufacturing is poised to play a pivotal role in driving sustainable solutions for biofuels, organic acids, terpenoids, and specialty chemicals. The future looks bright for yeast-based hosts to serve as a major contender for bio-based production of sustainable chemicals.

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Figure 1 was created with Biorender.com (https://BioRender.com/d99r330)

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Data availability

No new data were generated or analysed in support of this research.

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