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Review

Recent developments of oleaginous yeasts toward sustainable biomanufacturing

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Oleaginous yeast are remarkably versatile organisms, distinguished by their natural capacities to accumulate high levels of neutral lipids and broad substrate range. With recent growing interests in engineering non-model organisms as superior biomanufacturing platforms, oleaginous yeasts have emerged as promising chassis for oleochemicals, terpenoids, organic acids, and other valuable products. Advancement in systems biology along with genetic tool development have significantly expanded our understanding of the metabolism in these species and enabled engineering efforts to produce biofuels and bioproducts from diverse feedstocks. This review examines the latest technical advances in oleaginous yeast research toward sustainable biomanufacturing. We cover recent developments in systems biology-enabled metabolism understanding, genetic tools, feedstock utilization, and strain engineering approaches for the production of various valuable chemicals.

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Introduction

Microbial biomanufacturing is an attractive route for developing efficient platforms that enable the production of low-cost fuels and chemicals from sustainable resources to support a circular bioeconomy. While model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* have been extensively engineered to synthesize a wide array of products from diverse feedstocks, alternative microbial chassis with superior traits have emerged as highly promising platforms for efficient and cost-effective production. Among these, oleaginous yeasts stand out for their high lipid accumulation and exceptional metabolic versatility.

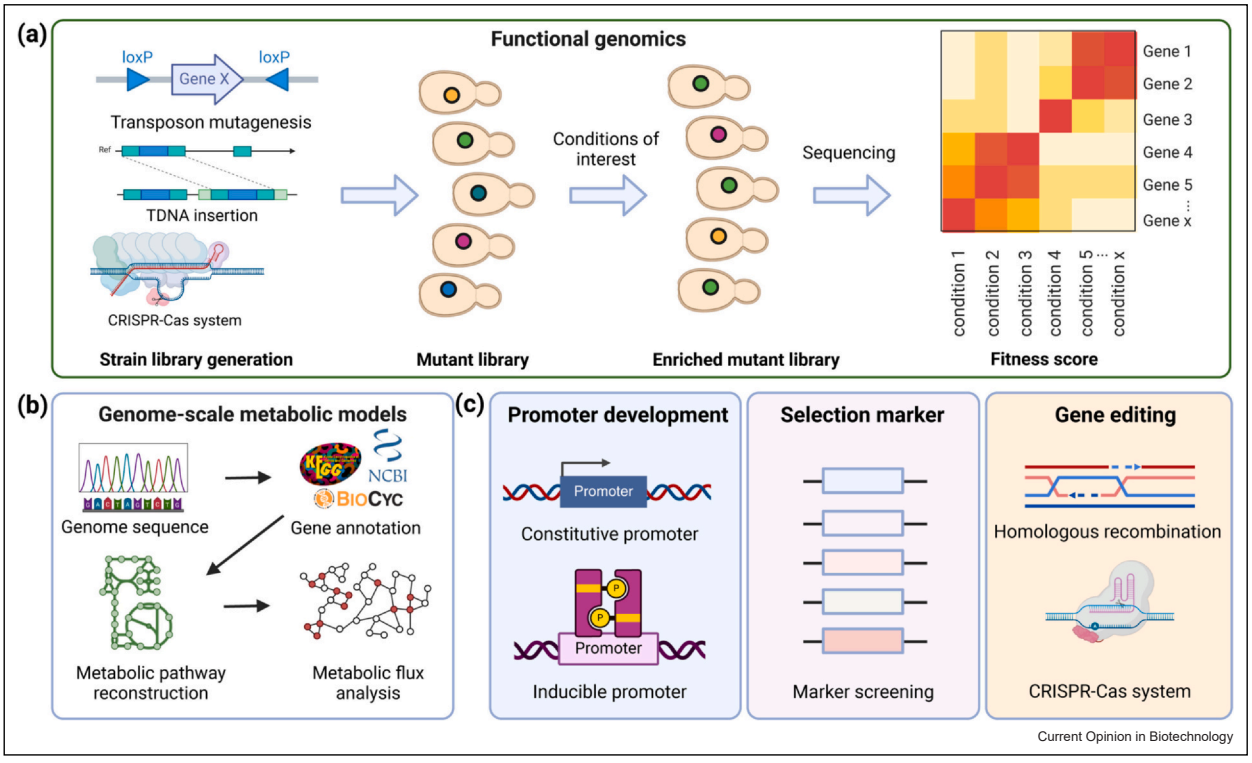
Oleaginous yeasts uniquely accumulate large lipid bodies, mainly triacylglycerols (TAGs), exceeding 20% of their dry weight, with a distinctive higher TAG-to-steryl ester ratio (~10:1) compared to non-oleaginous species (~1:1) [1]. These yeasts are highly versatile and can utilize diverse substrates and exhibit notable tolerance to variable pH, osmotic pressure, and common lignocellulose-derived inhibitors [2]. They have been engineered to produce diverse bioproducts, such as fuels, lubricants, polymers, plastics, and surfactants [3]. This review highlights recent progress in the understanding of metabolism, feedstock utilization, genetic engineering tool development, and bioproduct engineering in oleaginous yeasts.

Advances in understanding oleaginous yeast metabolism

The hallmark of oleaginous yeast is their lipid bodies triggered by nutrient starvation, particularly of nitrogen, phosphorus, and sulfur [4], enabling some strains to accumulate lipids to ~85% of their cell dry weight [5]. Despite their potential, a key challenge in utilizing oleaginous yeasts is the limited understanding of their unique metabolism. While the extensively studied model yeast *S. cerevisiae* provides a useful reference, oleaginous yeasts encompass diverse species spanning multiple clades within the Kingdom Fungi. These yeasts exhibit metabolic traits absent in *S. cerevisiae*, such as a uniquely high cytoplasmic acetyl-CoA (AcCoA) flux during nitrogen limitation, facilitated by ATP citrate lyase [6]. Nevertheless, advances in sequencing technologies, along with the integration of functional genomics, metabolic modeling, and multi-omics analyses, have significantly offered deeper insights into their complex metabolic networks.

Functional genomics has emerged as a powerful tool for elucidating gene essentiality and function under various growth conditions (Figure 1a). This approach has been

Figure 1



Recent advances in oleaginous yeast engineering toward sustainable biomanufacturing. **(a)** A functional genomics pipeline that starts with various methods to generate a library of mutants. The strain libraries are conditionally screened to enrich for viable mutants, and fitness scores are calculated to detect statistical significance of enriched mutant abundance versus a reference. **(b)** Construction and application of genome-scale metabolic models. With sequenced genomes, genes are annotated via reference genomes and databases to reconstruct a draft model that can be refined by phenotypic data. **(c)** Genetic tool development in oleaginous yeasts, including promoters, selectable markers, and gene editing ability. Figure created with Biorender.com.

exemplified by the construction of barcoded mutant libraries for *Y. lipolytica* and *R. toruloides* through techniques such as transposon mutagenesis, T-DNA insertions via *Agrobacterium tumefaciens*, and targeted CRISPR-Cas9-mediated disruptions [7–9]. These libraries have been instrumental in identifying conditionally essential genes and screening for traits, such as enhanced lipid accumulation, facilitated by lipid staining and cell-sorting methods. For instance, in *Y. lipolytica*, a highly functional Cas9 sgRNA library with over 94% genome-wide coverage revealed four novel genetic targets associated with increased lipid staining [9]. Moreover, lipid accumulation in *R. toruloides* was found to be regulated by genes with predicted functions in signaling cascades, protein modification, autophagy, and t-RNA thiolation; however, most of the identified targets remain methodically uncharacterized [8]. Expanding on these approaches, a whole-genome CRISPR interference (CRISPRi) library for *Y. lipolytica*, uncovered 14 novel genetic targets linked to inhibitor tolerance [10]. Such advancements offered critical insights into the genetic and regulatory networks underlying lipid metabolism and stress responses.

Genome-scale metabolic models (GEMs) provide a comprehensive framework for understanding metabolism (Figure 1b) yet exist for ~10 of the ~150 known oleaginous yeast species [11] — the most recent being *Lipomyces starkeyi* NRRL Y-11557 [12]. These models form the basis of computational tools that predict flux distributions and genetic perturbations to guide strain engineering and facilitate a rigorous understanding of metabolic pathways [11]. For instance, comparing GEM predictions with phenotypic growth data and multiomic analyses in *R. toruloides* suggested an alternative pathway for D-xylose and L-arabinose metabolism, proceeding via D-arabitol and D-ribulose to ribulose-5-phosphate. The suggested pathway diverges from the conventional route via D-xylulose-5-phosphate and was subsequently validated experimentally [13,14]. Given that D-xylose is a major component of lignocellulosic biomass, understanding its metabolism is critical to engineer strains

capable of efficient xylose utilization. Beyond mapping metabolic pathways, GEMs are instrumental in identifying genetic engineering strategies. For instance, a GEM-guided algorithm proposed overexpression targets in *Cutaneotrichosporon oleaginosus*, achieving 56 wt% lipids on a glycerol-defined medium when coupled with media optimization [15]. Furthermore, GEM and 13C-metabolic flux analyses were combined to probe TAG catabolism in *Y. lipolytica* [16]. However, traditional GEMs have limitations as they primarily predict reaction fluxes bounded by minimal physiological data [17]. Recent advancements incorporating enzyme constraints aim to simulate experimental phenotypes more accurately. These enhanced models, though, remain largely confined to well-studied organisms, such as *E. coli* and *S. cerevisiae* [18].

Tool development in oleaginous yeast

A robust genetic toolbox is foundational for advancing the oleaginous yeast engineering and should comprise transformation techniques, selectable markers, an array of promoters and terminators, episomal plasmids, efficient homologous recombination systems, CRISPR/Cas9 technologies, and rapid DNA assembly methods (Figure 1c) [19]. Recent studies have leveraged omics data to significantly expand available promoters and selectable markers in *C. oleaginosus* [20], *L. starkeyi* [21], and *R. toruloides* [22]. A notable advancement in *R. toruloides* identified and validated six nitrogen starvation–induced promoters via genomics and transcriptomics, which offer a means to regulate metabolic pathways based on nutrient availability [23]. To address the lengthy iterative design–build–test–learn cycles in strain development, the field increasingly relies on standardized synthetic biology toolkits comprising genetic parts and advanced DNA editing and assembly technologies [24]. Among these, DNA assembly strategies such as Golden Gate assembly have gained popularity due to the ease of one-pot multifragment assembly, even in the presence of repetitive sequences. Recently, this has been adopted in *R. toruloides*, alongside *Y. lipolytica* and *S. cerevisiae*, to streamline strain engineering [25].

The low endogenous homologous recombination efficiency in most oleaginous yeast poses significant challenges for precise genome editing and targeted strain engineering. The rapid advancements in clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated nuclease (Cas9) systems offer transformative solutions, unlocking versatile genetic editing tools for these organisms. While promising, beyond a few species such as *Y. lipolytica*, *R. toruloides* [26], *Aspergillus oryzae* [27], and *Candida viswanathii* [28], the implementation of CRISPR-Cas9 systems in most oleaginous yeasts is largely absent. Moreover, more sophisticated CRISPR tool variants such as CRISPR activation (CRISPRa), CRISPRi, and base editing have only been reported in *Y. lipolytica*. For instance, a CRISPR-Cas12a system in *Y. lipolytica*, guided by a deep learning algorithm for sgRNA selection, enabled an eightfold coverage genome-wide knockout functional genomics screen, facilitating further insights into oleaginous metabolism [29]. Expanding and adapting advanced CRISPR-based systems in a broader range of oleaginous yeasts holds immense potential for enabling rapid strain engineering, precise gene regulation, and multiplexed editing to accelerate metabolic engineering efforts.

In addition to genetic editing tools, uncovering the intricate transcriptional regulatory networks governing oleaginous yeast metabolism is key for effective strain engineering. Unfortunately, these networks remain significantly less understood in oleaginous yeasts compared to model organisms such as *S. cerevisiae* [30]. Addressing this gap, a web tool and database (YEASTRACT+) originally developed for *S. cerevisiae* [31], has recently been extended to pathogenic and nonconventional yeasts, including *Y. lipolytica* and *R. toruloides* [32]. This resource catalogs ~305,000 transcription factor–target pairings from 11 yeasts, supporting regulatory analysis, modeling, and prediction. It features a web tool that integrates these data with GEMs to refine engineering strategies, which currently applies only to *S. cerevisiae* and *C. albicans*. Complementary to this, a recently developed machine learning tool able to cluster and deconvolute an abundance of transcriptomics into meaningful modules was demonstrated in *Y. lipolytica* [33]. This approach identified 23 independently modulated and co-regulated gene sets, or iModulons, making *Y. lipolytica* the first eukaryote to utilize this technique initially developed for prokaryotes [34]. These iModulons typically represent genes regulated by a single transcription factor, which were further mapped to homologous *S. cerevisiae* regulons. Such insights can be harnessed for metabolic engineering by allowing researchers to either exploit or bypass specific regulatory pathways. However, current regulatory network models rely heavily on computational inferences derived from transcriptomic data, which often correlate poorly with *in vivo* protein levels at the population scale [35]. To robustly translate these predictions into effective engineering strategies, experimental validation will be essential.

Feedstock utilization

Feedstock cost is a significant factor in bioprocess economics, often accounting for over 50% of the minimum selling price of a product [36], unless cost-effective alternatives such as organic waste streams and lignocellulosic biomass are used [37]. Despite this potential, a recent survey of oleaginous yeast research reveals that only ~20% of studies utilized lignocellulosic

hydrolysates [1], with a notable study that screened 31 oleaginous yeasts for lipid production on corn stover hydrolysate [38]. This trend may stem from the toxicity of byproducts, such as furans, phenols, and organic acids, generated during biomass deconstruction, which inhibit microbial growth through mechanisms, such as reactive oxygen species generation, DNA fragmentation, redox imbalance, and enzyme inhibition [39]. Interestingly, some oleaginous yeast exhibit natural biodetoxification capabilities for these inhibitors, albeit at relatively low concentrations [40,41]. For instance, wild-type *R. toruloides* has been shown to degrade up to 80–100% of nonlethal concentrations of inhibitors, including furfural, 5-hydroxymethyl furfural, vanillin, syringaldehyde, vanillic acid, and levulinic acid [40]. Similarly, screening of five oleaginous yeast strains for growth, sugar utilization, and lipid accumulation in acid-treated wheat straw hydrolysate revealed that only two *Trichosporon cutaneum* strains could grow and accumulate lipids in the presence of phenolic aldehydes after furan aldehydes and organic acids were removed [41]. The exact biodetoxification mechanisms in these strains remain unclear; thus, future studies are needed to identify the responsible pathways and associated genes.

Alternatively, adaptive laboratory evolution (ALE) is a powerful approach to improve microbial tolerance to pretreatment inhibitors through continuous propagation under selective pressure (Figure 2). For instance, *R. toruloides* evolved on methanol exhibited increased tolerance to common furan aldehydes and acids commonly seen in lignocellulosic hydrolysates. The increased tolerance was attributed to decreased membrane permeability and an altered membrane structure resistant to zymolyase and propidium iodide staining, though the genetic basis remains unclear [42]. In addition to solely focusing on improving tolerance, simultaneous enhancement in lipid production can be achieved with adapted ALE protocols. *T. cutaneum* that underwent ultracentrifugation and inhibitor-spiked hydrolysate adaptation evolved increased cell wall integrity, sixfold higher lipid production, and enhanced tolerance to furan aldehydes, linked to upregulation of aldehyde and alcohol dehydrogenases of the phenolic aldehyde bioconversion pathway [43]. In addition to conventional pretreatment methods, ionic liquids (ILs) offer a novel green alternative to deconstruct biomass, but their residual presence can lead to toxicity issues [44]. To address this, *Y. lipolytica* was evolved for high IL tolerance, achieving 18% (v/v) tolerance to 1-ethyl-3-methylimidazolium acetate [44]. Transcriptomic analysis and gene coexpression studies identified two genes involved in mitotic cell division, chromatin segregation, and Golgi vesicle transport as key contributors to this phenotype, which was successfully recapitulated through reverse engineering. Further studies are needed to fully elucidate the mechanisms of the enhanced tolerance to inform engineering efforts toward physiology changes and metabolic shifts to enable higher inhibitor tolerance.

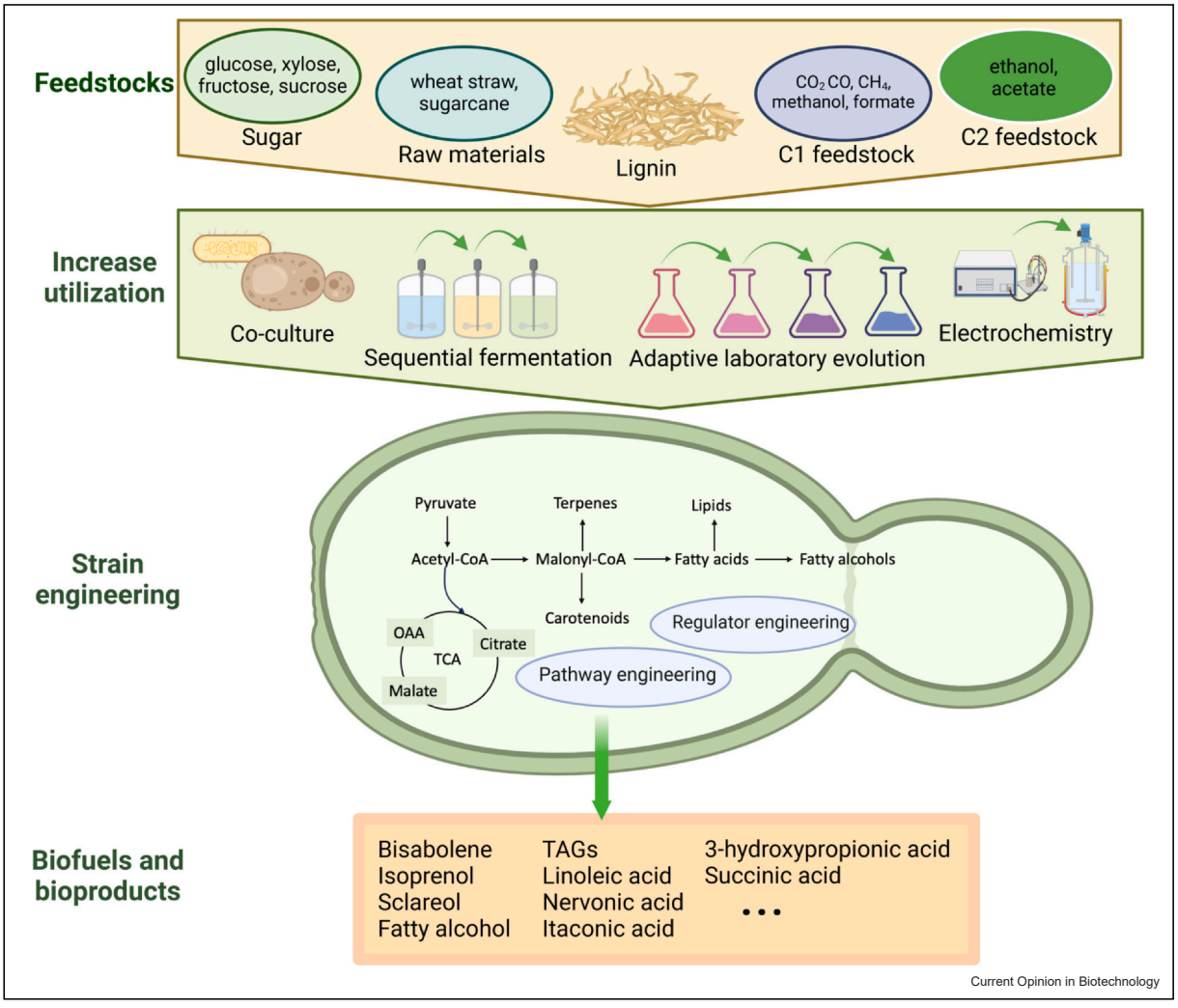
While some oleaginous yeasts are known to consume a variety of carbon sources present in hydrolysates, a recent survey of 1200 yeast strains for lipid production identified only 12 strains capable of co-utilizing multiple carbon sources [45]. These include glucose and xylose co-utilization strains such as *Pseudozyma hubeiensis* [46], xylose and cellobiose co-utilization strains such as *C. curvatus* [47], glucose and acetic acid co-utilization strains *R. toruloides* and *R. mucilaginosa* [48], and acetate, propionate, and butyrate co-utilization strain *T. cutaneum* [49]. These findings warrant further research into alleviating carbon catabolite repression and engineering substrate-specific transporters to enhance simultaneous carbon utilization.

In addition to lignocellulosic feedstocks, atypical carbon sources for oleaginous yeast such as C1 feedstocks (methanol, CO_2, formate) have been explored through various approaches. These include introducing C1 metabolic pathways directly [50], leveraging electrochemical catalysis to convert C1 feedstocks into bioavailable intermediates [51], and employing sequential fermentation [52,53] or co-culturing with C1-metabolizing microbes [54,55]. These approaches leverage well-characterized C1 metabolic pathways and established electrochemistry, and have significantly expanded the substrate range of oleaginous yeasts toward versatile and low-cost feedstocks.

Strain engineering of oleaginous yeast to produce biofuels and bioproducts

Research on oleaginous yeasts has extensively leveraged their naturally high lipid biosynthesis capacity to produce various fatty acids and fatty acid–derived products [56,57]. Fruitful strategies commonly used to improve titer, rate, and yield include overexpressing genes supplying AcCoA and malonyl-CoA precursors [56], blocking β-oxidation to prevent product degradation [58], and optimizing nicotinamide adenine dinucleotide phosphate supply (Figure 2) [59]. Nitrogen limitation plays a pivotal role in lipid synthesis [60], and engineering nitrogen metabolism regulators can redirect carbon flux toward lipid accumulation [61]. For instance, the GATA-family transcription factor GAT1 is known to regulate catabolite repression as well as nitrogen response and utilization. Regulatory modifications such as GAT1 overexpression in *Saitozyma podzolica* [62] and deletion of SNF1 in *L. starkeyi* [63], have significantly enhanced lipid production. Beyond directly engineering pathway regulators, production can also benefit from conditionally activated gene expression. For example, a lipogenic phase–activated promoter was identified via transcriptomics and was used to overexpress two β-

Figure 2



Schematic overview of various feedstocks, strategies to enhance feedstock utilization, and metabolic engineering of oleaginous yeasts to produce biofuels and bioproducts. Figure created with Biorender.com.

ketoacyl-CoA synthases (KCS) to enhance the production of nervonic acid (NA). Alongside with additional multitiered strain optimization, the resulting strain produced 44.2 g/l NA at 0.23 g/l/h, the highest titer and rate reported to date [57].

Beyond fatty acid–derived products, the naturally high AcCoA flux [6] in oleaginous yeasts has been widely exploited to engineer a broad array of biofuels and bioproducts, such as isopropanol [64], terpenes [65,66], triacetic acid lactone [67], polyhydroxybutyrate [68], and 3-hydroxypropionic acid [69]. For instance, *Y. lipolytica* was engineered to produce the diterpene alcohol sclareol [66]. A literature-high titer of 12.9 g/l in a 5 l bioreactor was achieved by scaffold-free enzyme assembly, screening and engineering of heterologous (13*E*)-8α-hydroxylabden-15-yl diphosphate LPP and sclareol synthases, along with overexpression of upstream rate-limiting steps. Because lipid accumulation competes for the AcCoA pool, two predominant strategies to enhance pools for alternative pathways are commonly used, namely, lipid biosynthesis downregulation [65] and/or enhancing endogenous lipid degradation [70]. A recent study that leverages both strategies focuses on β-farnesene production from waste cooking oil in *Y. lipolytica*. A high titer of 31.9 g/l was achieved with compartmentalizing the mevalonate pathway into peroxisomes, enhancing β-oxidation, attenuatingglucose intracellular lipid synthesis, as well as protein engineering of farnesene synthase [70].

Finally, oleaginous yeasts have been engineered to produce various organic acids including citric acid (111 g/l), α-ketoglutarate (56.5 g/l), succinic acid (160 g/l), itaconic acid (4.6 g/l), as well as D-arabitol (49 g/l),

Table 1

Representative biofuel and biochemical productions in oleaginous yeasts reported in recent years.

Oleaginous yeasts	Carbon source	Product of interest	Strategy	Titer/yield/ productivity	Refs
R. toruloides	Methanol, acetic acid, formic acid, furfural	Lipid	ALE	36.2% of the dry cell biomass	[42]
	Glucose	Fatty alcohols	Gene overexpression and Cas9-mediated gene deletions	3.7 g/l	[56]
	Glucose and corn steep liquor	Nervonic acid	Improving the biosynthesis of VLCFAs during lipogenic phase	44.2 g/l	[57]
	Glucose	Triacetic acid lactone	Pathway engineering	28 g/l	[67]
	Corn stover hydrolysate	3-hydroxypropionic acid	Transporter overexpression & 3HP catabolic gene deletion	45.4 g/l	[69]
	Xylose	D-arabitol	Media optimization	49 g/l	[71]
	Glucose	Indigoidine	Pathway overexpression and media optimization	18 g/l	[72]
Y. lipolytica	Methanol	Succinic acid	Systematic metabolic engineering	0.92 g/l	[50]
	Acetate, formate	β-farnesene	CO_2 electrocatalysis	14.8 g/l	[51]
	Crude glycerol	Isopropanol	Integrating exogenous genes	1.6 g/l	[64]
	Cooking oil waste	β-farnesene	Peroxisome compartmentalization of the mevalonate pathway	31.9 g/l	[70]
	Glucose	High-oleic acid TAGs	Pathway engineering	56 g/l	[58]
	Cooking oil waste	bisabolene	Peroxisomal pathway engineering	15.5 g/l	[65]
	Glucose	Sclareol	Pathway & protein engineering	12.9 g/l	[66]
C. oleaginous	Corn Stover	Lipid	ALE	33.8 g/l	[43]
	Acetate, propionate, butyrate	TAGs	Media optimization	7.5 g/l	[49]
C. viswanathii	Glucose, Dodecane	Dedecanedioic acid	Overexpression of rate-limiting steps	224 g/l	[28]
R. glutinis	Glucose	Lipids	Heterologous malic enzyme overexpression	40 wt%	[59]
P. hubeiens	Glucose, Xylose, L-arabinose	Lipid	Co-fermentation	1.8 g/l	[45]
R. paludigena	Glucose	Polyol esters of fatty acids	Fed-batch process and media optimization	110 g/l	[73]
R. mucilaginosa	Synechocystis sp. PCC 6803 biomass	Carotenoids	Media optimization	220 mg/g biomass	[53]
R. toruloides and A. falcatus co-culture	Palm Oil	Lipids	Co-cultivation optimization	15.4 mg/l/d	[55]

VLCFAs, very long chain fatty acids.

erythritol (80.6 g/l), and indigoidine (18 g/l) at high titers [3] [71,72]. These studies demonstrate the product portfolio diversity in oleaginous yeasts, and suggest great opportunities to explore and leverage various abundant precursor metabolites to produce a wide range of products in these species. Table 1 compiles representative biofuel and biochemical productions in oleaginous yeasts reported in recent years.

Conclusions and future perspectives

Substantial progress has been made in oleaginous yeast research, particularly in the last decade, that has significantly advanced the understanding and development of these species toward manufacturing a wide range of biofuels and bioproducts at high titers, rates, and yields. Among oleaginous yeasts, *Y. lipolytica* has been most extensively explored and has commercialized products [74]. Other oleaginous yeasts such as *R. toruloides* and *L. starkeyi* have attracted increasing academic and industrial interests and are not far behind. So far, microorganisms are considered oleaginous if they produce high levels of intracellular neutral lipids [75]; however, such definition does not include species with high capacity to produce lipids in forms other than TAGs. Broadening the definition of oleaginous yeasts to encompass diverse lipid classes (produced intra- or extra-cellularly), such as glycerophospholipids, sphingolipids, sterols, fatty acids, prenols, saccharolipids, polyketides, may help to reveal their versatility and identify promising candidates for the production of oleochemicals [75]. For instance, *Starmerella bombicola* and *Rhodotorula paludigena BS15* extracellularly produce sophorolipids and polyol esters of fatty acids at 200 g/l and 120 g/l, respectively, which demonstrate their industrial potential [73,76].

Cost-competitive bioproducts to petroleum-based equivalents are one of the several hurdles facing their industrialization [77,78]. To bridge the gap between academic research and industrial application, integration of techno-economic and life-cycle analyses (TEA & LCA) into early-stage projects is critical to align proof-of-concept studies with commercial feasibility [79]. Such analyses are crucial for evaluating optimal process conditions, major cost drivers, policy incentives, and setting target strain performance metrics to guide research efforts. While TEA and LCA are immensely helpful, they depend on technical assumptions that rely on performance data at scale. Thus far, most studies occur at the bench scale and scale-up remains underrepresented in the literature [1]. Periodic TEA/LCA parametrization with scale-up data is needed to provide more realistic model inputs, as fermentation metrics (e.g., titers, rates, yields) will differ due to scale-dependent differences in mixing efficiency, gas transfer rates, shear stress, and others [80], as well as fermentation operation modes (batch, fed-batch, continuous). The wide adoption and routine utilization of these analyses will help enable an increased likelihood of successful product development, and bring oleaginous yeasts to broad industrial adoption toward a circular economy.

Author contributions

Paul A. Adamczyk: Conceptualization, Writing – original draft, Writing – review & editing the manuscript, Visualization. **Tian Jiang**: Writing – original draft, Writing – review & editing the manuscript, Visualization. **Karuna Jetty**: Writing – original draft, Writing – review & editing the manuscript. **Vijaydev Ganesan**: Writing – original draft, Writing – review & editing the manuscript. **Di Liu**: Conceptualization, Supervision, Writing – original draft, Writing – review & editing the manuscript, Visualization, Funding acquisition.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

Nothing declared.

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