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Review article

Expanding biotechnological applications of *Yarrowia lipolytica*: Key advances in the past decade

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ABSTRACT

Yarrowia lipolytica is a non-conventional yeast with innate oleaginous metabolism and unusual tolerance for hydrophobic substrates, positioning it as a prime chassis for waste-enabled precision fermentation. In this review,

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Abbreviations: 4-CL, 4-coumaryl ligase; 6PGDH, 6-phosphogluconate dehydrogenase; 6PGL, 6-phosphogluconolactonase; 6-PGL, 6-phosphogluconolactone; 6-PL, 6-phosphogluconate; ACC1, acetyl-CoA carboxylase; ACL1, ATP-citrate lyase; ACO1, peroxisomal aconitase; ACO2, mitochondrial aconitase; ACS1, acetyl-CoA synthase; ADH, alcohol dehydrogenase; ALD, aldehyde dehydrogenase; ARE1, sterol acyltransferase; AS, glycosyltransferase; CA, citric acid; CAD, cis-aconitate decarboxylase; CAR, carboxylic acid reductase; CARB, phytoene dehydrogenase; CARRB, phytoene synthase / lycopene cyclase; CCD2, carotenoid-cleaving dioxygenase; CHI, chalcone isomerase; CHS, chalcone synthase; CIT1, mitochondrial citrate synthase; CIT2, peroxisomal citrate synthase; CNS1, oxidoreductase / dehydrogenase enzyme complexes; CNS2, metal-dependent phosphohydrolases; CPR, cytochrome P450 monooxygenase; CPT, carnitine palmitoyltransferase; CrtZ, β-carotene hydroxylase; DAHP, 3-deoxy-D-arabino-heptulosonic acid 7-phosphate; DAHPS, 3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthase; DAK1, dihydroxyacetone kinase; DGAT1, diacylglycerol acyltransferase; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; DHQ, dihydroquercetin; DMAPP, dimethylallyl pyrophosphate; DPP1, diphosphate phosphatase; ERG1, squalene monooxygenase; ERG10, acetoacetyl-CoA thiolase; ERG12, mevalonate kinase; ERG13, hydroxymethylglutaryl-CoA synthase; ERG19, diphosphomevalonate decarboxylase; ERG20, farnesyl diphosphate synthase; ERG8, phosphomevalonate kinase; ERG9, squalene synthase; EYD1, erythritol dehydrogenase; F3H, flavonoid-3-hydroxylase; FAA, fatty acyl-CoA synthetase; FAD2, fatty acid desaturases; FAR, fatty acyl-CoA reductase; FAS1, fatty acid synthetase; FBA, fructosebisphosphate aldolase; FPP, farnesyl pyrophosphate; FS, farnesene synthase; FUM1, fumarase; G6PDH, glucose-6-phosphate dehydrogenase; GGOH, geranylgeraniol; GGPP, geranylgeranyl pyrophosphate; GGS1, geranylgeranyl pyrophosphate synthase; GLK1, glucokinase; GND1, 6-phosphogluconate dehydrogenase; ZWF1, glucose-6-phosphate dehydrogenase; GPI, glucose-6-phosphate isomerase; GPP, geranyl pyrophosphate; GUT1, glycerol kinase; GYC1, glycerol dehydrogenase; HK, hexokinase; HMG-CoA, hydroxymethylglutaryl-CoA; HMGR, hydroxymethylglutaryl-CoA reductase; ICL1, isocitrate lyase; IDH2, mitochondrial isocitrate dehydrogenase; IDI1, isopentenyl-diphosphate delta-isomerase; IDP, isocitrate dehydrogenase; IPP, isopentenyl pyrophosphate; Iso-CA, isocitric acid; KGDH, ketoglutarate dehydrogenase; KS, ketoacyl synthase; LIP2, lipases; LIS, linalool synthase; LPP1, lipid phosphate phosphatase; LS, limonene synthase; LSC1, succinate-CoA ligase; LRO1, acyltransferase; MAS, multifunctional amyrin synthase; MDH1, mitochondrial malate dehydrogenase; MDH3, peroxisomal malate dehydrogenase.; MFE1, multifunctional enzyme; MLS, malate synthase; MTT, mitochondrial tricarboxylic transporter; NAR, (2*S*)-naringenin; NDPS1, neryl diphosphate synthase; NPP, neryl pyrophosphate; OAA, oxaloacetic acid; PAL, phenylalanine ammonia lyase; PDC1, pyruvate dehydrogenase complex; PEP, phosphoenolpyruvate; PFK, phosphofructokinase; PGM1, phosphoglucomutases; PHA2, prephenate dehydratase; PK, pyruvate kinase; POT1, peroxisomal thiolase; POX1-6, six different acyl-CoA oxidases; PT1, prenyltransferase; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate; SA, succinic acid; SDH, succinate dehydrogenase; TAL, tyrosine ammonia lyase; TKT, transketolase; TPI, triosephosphate isomerase; TRP2, anthranilate synthase; TYR1, prephenate dehydrogenase; UbiC, chorismite lyase; α-KG, α-ketoglutarate.

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Precision fermentation
Synthetic biology

we consolidate advances associated with Yarrowia over the past decade into a coherent, trait-centered framework that links biological capabilities to manufacturing performance. We studied genetic toolkits, adaptive laboratory evolution and mating/fusion for strain hardening and phenotype expansion, and combinatorial metabolic engineering strategies (push-pull-block lipid routing, stage- and compartment-specific expression, export engineering) that together improved production titer, rate, and yield across various scales. By organizing metabolic products based on core pathways, namely, the tricarboxylic acid cycle, mevalonate pathway, pentose phosphate pathway, and fatty acid biosynthesis, and pairing each class with the specific chassis edits that enabled leading titers, we provide actionable guidance for target selection and de-risked development. Finally, we propose a roadmap leveraging emerging approaches, AI-guided design, intensified bioprocessing, and precision co-cultures, as key enablers of a scalable, circular bioeconomy with Y. lipolytica as a platform strain.

1. Introduction

Metabolic engineering comprises optimizing native metabolic pathways and regulatory networks or building foreign metabolic pathways through genetic, molecular, or combinatorial methods to produce specific compounds (Zhu and Jackson, 2015). The primary objective of metabolic engineering is to establish a ‘cell factory’ capable of efficiently producing targeted molecules for a wide range of industrial applications. This underscores the practical significance of the field (Bailey, 1991; Chen and Nielsen, 2013). Success in metabolic engineering hinges on several crucial factors. These include a thorough understanding of the metabolic pathway, the selection of robust and safe microorganisms as hosts, the maintenance of co-factor balances and regulatory networks, the use of appropriate transformation methods, and the implementation of strategies to minimize the formation of toxic products and intermediates that can inhibit the process.

For the past few decades, *Yarrowia lipolytica* has received massive attention as one of the hosts in metabolic engineering, specifically for the bioproduction of high-value chemicals derived from fatty acids (FA), acetyl-CoA, and lipids (Bankar et al., 2009; Liu et al., 2015a, 2015b; Thevenieau et al., 2009). This species has emerged as a model non-conventional oleaginous yeast in the diverse studies involving protein secretion pathways, lipid degradation and accumulation, dimorphism, as well as peroxisome biogenesis. In comparison with other microorganism (*e.g.* bacteria and some other type of yeasts), *Y. lipolytica* is recognized as “generally regarded as safe” (GRAS) microorganism (Beopoulos et al., 2009; Fukuda, 2013; Nicaud, 2012). Being classified as an oleaginous yeast, this strain possesses an exceptional ability to store large amount of lipid (up to 30-50% of its cell dry weight), making it as one of the promising platforms for the production of biofuels and other fatty acids-derived products *via* Triacylglycerol (TAG) biosynthesis pathway (Madzak, 2021). *Y. lipolytica* also exhibits excellent physiological characteristic compared with the other microorganism due to its high tolerance towards environmental stresses, including its robust growth in low pH, low temperatures, and high salt concentrations, as well as the ability to grow on a broad scope of renewable (waste-based) and hydrophobic substrates (Park and Ledesma-Amaro, 2023). Given its distinctive metabolic features and the recent advancements in metabolic engineering, additionally, *Y. lipolytica* holds a significant potential for the cost-effective and sustainable production of a wide range of novel products and high-value metabolites. Compared to these traditional platforms, this strain serves as an effective platform for

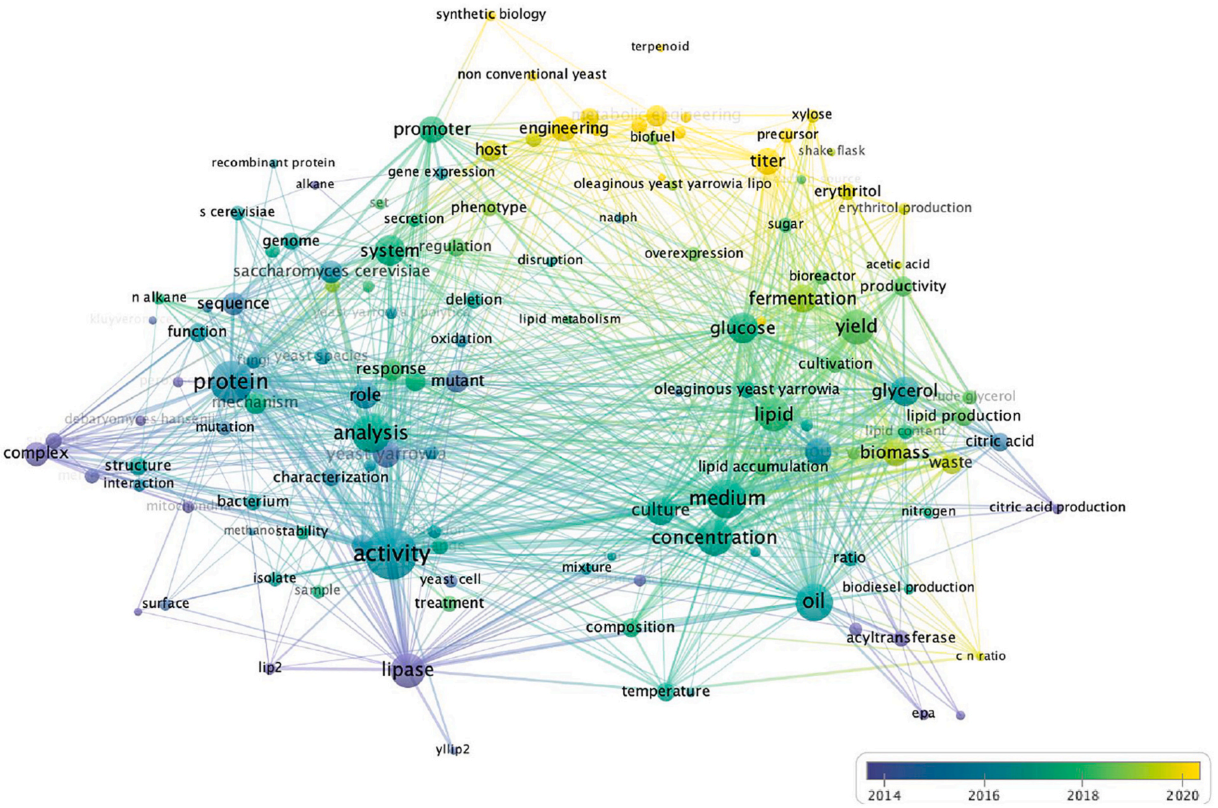


Fig. 1. Network map depicting commonly occurring terms in the titles and abstracts of *Yarrowia lipolytica* journal articles (2005–2025), based on 2410 articles retrieved from the Europe PMC database.

heterologous protein secretion and expression, thus has been tremendously used in many applications in the biotech-based industries (Madzak, 2015).

To understand the key aspects of *Y. lipolytica* in biotechnological research and development, VOSViewer v.1.6.20 software (van Eck and Waltman, 2009) was used to retrieve journal articles from Europe PMC database. 2410 journal articles were retrieved for the period from 2005 to 2025 that had mentioned '*Yarrowia lipolytica*' in their title or abstract. VOSViewer tool was used to build and visualize the network of keywords in the abstract and title of these articles. The nodes in the network represent keywords, while the edges represent co-occurrences. Fig. 1 shows most of the work on *Yarrowia lipolytica* focused on general molecular and basic biology until 2015. Subsequently, there was a shift in the focus towards metabolic engineering and biotechnology. If we take a closer look at the two major nodes of protein and fatty acid, we can observe that the majority of the work is related to both. For protein, it is mainly deciphering the basic cellular and molecular mechanisms, studying the various elements like promoter, transporter, *etc.*, and more recent works focusing on host engineering and fatty acid production. For the fatty acid node, the earlier works focused on elements like lipase, acetyltransferase, n-alkane production, substrate specificity, *etc.* In contrast, recent work has focused on metabolic engineering, feedstock utilization, waste, microbial production, *etc.*

The application of *Y. lipolytica* on the industrial scale has been extensively covered by Jackson and Amaro's group in 2015 and 2023, respectively (Park and Ledesma-Amaro, 2023; Zhu and Jackson, 2015). Hence, this article will comprehensively focus on *Y. lipolytica* and provide insights into its current biological understanding and achievements in metabolic engineering from the perspective of biological traits. This includes, but is not limited to, its application in bioremediation, adaptive laboratory evolution, and its capability to produce single-cell proteins and high-value biochemicals for the past decade.

2. Genetic toolkits and methods for *Yarrowia lipolytica*

2.1. Yeast promoters, terminators, and DNA regulatory elements

The ability to tune gene expression is key for obtaining optimal amounts of the encoded enzyme and maximizing the output of the genetic circuit. One of the most important determinants of gene expression is the choice of the promoter. While a strong constitutive promoter might be suitable for a non-toxic protein, an inducible/stationary phase/weak constitutive promoter is more appropriate for proteins that inhibit cell growth. In a multi-gene biosynthetic gene cluster, the relative expression of different genes is crucial for achieving high product titres. Balancing the expression of various genes in a cluster is important for maximizing the metabolic flux towards the end product and reducing the toxic side effects of overexpression. Work from several synthetic biology hosts has indicated that no one promoter works best for all genes. In practice, researchers scan through diverse promoter combinations for their cluster genes and choose one that works best.

A typical eukaryotic promoter consists of a core promoter and an Upstream Activating Sequence (UAS). The core promoter consists of the TATA box, transcription start site (TSS) and the 5' untranslated region and serves as a binding platform for RNA Pol II and the general transcription factors. Specific transcriptional activators bind to UAS and promote the initiation of RNA Pol II-mediated transcription. Research in the last 30 years or so has generated a large set of promoters that are different in their strength and regulatory features for the *Yarrowia* synthetic biology toolbox. Table 1 lists the various promoters for *Y. lipolytica* reviewed in this section, along with their properties and source publication. Promoters can be classified into either constitutive or inducible. The constitutive/inducible promoters can be further categorized as endogenous or synthetic.

Table 1
List of Promoters and Terminators in *Yarrowia lipolytica.*

No	Element	Feature	Ref.
1	TEF1p	Constitutive promoter	(Müller et al., 1998)
2	FBA1p		(Hong et al., 2012)
3	TDH2p		(Hong et al., 2012)
4	GPM1p		(Hong et al., 2012)
5	GPATp		(Xue et al., 2013)
6	GPDp		(Xue et al., 2013)
7	$\mathrm{FBA1_{INP}}$		(Hong et al., 2012)
8	$\mathrm{GPD_{INP}}$		(Xue et al., 2013)
9	YAT1p	Active during lipogenesis and inactive during growth phase	(Xue and Zhu, 2006)
10	p1(YALI0B16522g)		(Kamineni et al., 2020)
11	p3(YALI0A04631g)		(Kamineni et al., 2020)
12	p4(YALI0A12815g)		(Kamineni et al., 2020)
13	p5(YALI0B19800g)		(Kamineni et al., 2020)
14	hp1-4d	Synthetic constitutive promoter	(Madzak et al., 2000)
15	$\mathrm{UAS1_{XPR2}}$ (1-32 copies)-LEU2pmin		(Blazeck et al., 2011)
16	$\mathrm{UAS1_{XPR2}}$ (1-32 copies)-TEF1pmin		(Blazeck et al., 2011)
17	$\mathrm{UAS1_{XPR2}}$ (3 copies)-EXP1pmin		(Zhao et al., 2021)
18	XPR2p	Endogenous promoter inducible by peptone	(Madzak et al., 2000)
19	POX2p	Endogenous promoter inducible by fatty acids and repressed by glucose and glycerol	(Juretzek et al., 2000)
20	POT1p		(Juretzek et al., 2000)
21	EYK1p	Endogenous promoter induced by oleic acid, erythritol, and erythrulose and repressed by glycerol and glucose	(Trassaert et al., 2017)
22	PMT1p–PMT2p, PMT3p–PMT4p and PMT1p–PMT2p	Endogenous bi-directional promoters induced by Copper sulphate	(Xiong and Chen, 2020)
23	CTR1p and CTR2p	Endogenous promoters repressed by Copper sulphate	(Xiong and Chen, 2020)
24	$\mathrm{UAS1_{EYK1}}$ (3 copies)-EYK1pMIN	Synthetic promoter inducible by erthritol/erythrose	(Trassaert et al., 2017)
25	$\mathrm{UAS1_{XPR2}}$ (4 copies)-EYK1pMIN		(Trassaert et al., 2017)
26	$\mathrm{URS1_{POX1}}$ (tandem copies)-TEF1pMIN	Synthetic promoter inducible by fatty acid	(Shabbir Hussain et al., 2017)
27	XylO-TEF1pMIN	Synthetic promoter inducible by xylose	(Wei et al., 2020)
28	CarO-promoter	Synthetic promoter inducible by green light	(Zhang et al., 2021)
29	C120-CYCpMIN	Synthetic promoter inducible by blue light	(Wang et al., 2022b)
30	*Sc* CYC1t	Terminator	(Blazeck et al., 2011)
31	*Yl* XPR2t		(Hong et al., 2012)
32	*Yl* LIP2t		(Nicaud et al., 2002)
33	SYNTH1t – SYNTH30t	Short synthetic Terminators	(Curran et al., 2015)

2.1.1. Constitutive-endogenous promoters

Promoters of highly expressed housekeeping genes have been a popular choice for achieving strong constitutive expression of the target gene. Some of the widely used promoters include TEF1p (Translation Elongation Factor 1 α) (Müller et al., 1998), FBA1p (Fructose-bisphosphate aldolase) (Hong et al., 2012), EXP1p (Export Protein) (Xue et al., 2013), GPM1p (Phosphoglycerate mutase) (Hong et al., 2012), GPD1p (Glycerol 3-Phosphate dehydrogenase) (Xue et al., 2013), and GPATp (Glyceraldehyde 3-phosphate dehydrogenase) (Blazeck et al., 2011) (Table 1). Some studies have compared the activities of these promoters by fusing them to a reporter gene like galactosidase or GFP (Juretzek et al., 2000; Wong et al., 2017). While the list of promoters grows continually, it has become necessary to compare the strength of all promoters in parallel in a single system.

The presence of an intron in the promoter sequence boosts gene expression in eukaryotic cells *via* an effect referred to as Intron-mediated gene enhancement. Introns could work by increasing the transcription rate, facilitating nuclear export, or enhancing mRNA stability/translation. About 14.5% of *Yarrowia* genes contain an intron. The presence of an intron in the TEF1-promoter sequence caused a 17-fold increase in expression in comparison to the intron-less promoter (Tai and Stephanopoulos, 2013). Overexpression of the triacylglycerol synthase gene (LRO1) from the TEF-Intron promoter (TEF1inp) boosted lipid production in the Po1g strain without affecting cell growth (Amalia et al., 2020). However, the authors did not compare the expression with that from an intron-less TEF1p (Amalia et al., 2020). FBA1inp was found to be five-fold stronger than the intron-less FBA1p (Hong et al., 2012). The presence of the intronic FBA1 sequence also boosted the transcriptional activity of the GPM1p, suggesting that the intron-mediated enhancement is transferable to other promoters (Hong et al., 2012). GPD1inp was used in the landmark paper describing the production of omega fatty acids in *Yarrowia lipolytica* (Xue et al., 2013). However, no comparison was made with the intron-less promoter versions. A more recent paper showed that the presence of the intron in TEF1p, EXP1p, and TDH1p boosted the expression of the GFP reporter compared to the intron-less versions (Cui et al., 2021).

Metabolites such as fatty acids / triacylglycerols (TAG) are produced during the stationary phase when the nitrogen levels drop. Therefore, a common production strategy for lipids is accumulating biomass during the growth phase, followed by producing lipids during the stationary phase. It is desirable to have promoters that are active either in the growth phase or the stationary phase. The promoter of the Ammonium Transporter gene (YAT1) is active only during the lipogenesis stage. Four promoters, p1(YALI0B16522g), p3(YALI0A04631g), p4 (YALI0A12815g) and p5 (YALI0B19800g), were active during the growth phase, but silent during the lipogenesis phase (Kamineni et al., 2020). The use of such growth-stage-specific promoters can greatly help maximize the production of toxic lipids and metabolites during the growth phase.

2.1.2. Constitutive-synthetic promoters

The Upstream Activator Sequences form landing pads for transcriptional activators, which recruit the RNA Polymerase II and the general transcription factors to the core promoter sequence. By increasing the copies of the UAS, the strength of the native promoter can be progressively increased. The first synthetic promoter in *Y. lipolytica* was generated using the UAS element of the XPR2 gene, which encodes an extracellular alkaline protease (Madzak et al., 2000). One to four copies of the $UAS1_{XPR2}$ sequence were added to the XPR core promoter to generate 4 synthetic promoters: hp1d-hp4d (Madzak et al., 2000). Apart from being stronger than the native promoters, they aid in separating cellular growth from metabolite production and have, therefore, been extensively used in synthetic biology. Similar approaches were accomplished by fusing multiple copies of the $UAS1_{XPR2}$ repeats to the core promoter sequences of either Leu2 or Tef1 (Blazeck et al., 2011). 1-32 copies of $UAS1_{XPR2}$ were fused to the minimal LEU2 promoter, and the hybrid promoters with more than 8 copies of UAS were more active than the TEF1 promoter (Blazeck et al., 2011). Likewise, the fusion of 8 or 16 copies of $UAS1_{XPR2}$ to the TEF1 promoter increased the activity more than the basal TEF1 promoter (Blazeck et al., 2011). The promoter generated by a fusion of three copies of the $UAS1_{XPR2}$ sequence to the core EXP1 promoter sequence was not as active as a similar fusion of three copies of the UAS_{XPR2} to TEF1 or Leu2 core promoter sequences (Zhao et al., 2021). However, when these promoters were used to express *S. cerevisiae* ARO10 (Phenylpyruvate decarboxylase) to produce isoamyl alcohol, the UAS_{EXP1m} was the most efficient (Zhao et al., 2021). It is possible that the presence of the ARO10 ORF increased the strength of the UAS_{EXP1} promoter. However, the authors did not compare the ARO10 mRNA levels in the different strains (Zhao et al., 2021). Taken together, these results suggest that the strength of the *Yarrowia* promoters is restricted by the activity of the UAS, which can be overcome by increasing the number of copies of UAS.

2.1.3. Inducible endogenous promoters

One of the first inducible promoters identified was that of XPR2, which encodes an extracellular protease (Davidow et al., 1987). XPR2p is peptone-inducible, and its UAS sequence was used to construct several synthetic promoters, as described above. Due to the oleaginous nature of *Yarrowia*, several lipogenic promoters have been explored for robust expression. Promoters of peroxisomal acyl-CoA oxidase 2 (POX2) and peroxisomal 3-ketoacyl-thiolase (POT1) were found to be inducible by fatty acid and repressed by glucose and glycerol. ICL1p was also inducible by fatty acid but was not repressed by glucose and glycerol (Juretzek et al., 2000). Promoter strengths of 11 lipogenic genes (GAP, ACL2, ICL, IDH2, FAS1, DGA1, FAS2, ZWF1, POX4, ACC and IDP2) were compared with that of TEF1p (Wong et al., 2017). The TEF1p was the strongest, and the strength of 11 lipogenic promoters decreased in the sequence of their gene names mentioned above, displaying 0.7 to 29.7% of TEF1p activity (Wong et al., 2017). Another inducible promoter is the EYK1p (erythrulose kinase), which is activated by oleic acid, erythritol, and erythrulose and repressed by glycerol and glucose (Trassaert et al., 2017). Subsequent research showed that EYK1p is a bi-directional inducible promoter as it controls the expression of both EYK1 and the adjacent gene EYL1(Erythrulose-4-phosphate isomerase) (Vidal et al., 2023). Three sets of bi-directional copper-inducible promoters (PMT1p-PMT2p, PMT3p-PMT4p and PMT1p-PMT2p) were identified by examining the upstream regulatory sequences of 6 metallothionein genes (MT-1 to MT-6) (Xiong and Chen, 2020). PMT2p and PMT6p were the most active and were comparable to the strong TEF1p in the presence of copper sulphate (Xiong and Chen, 2020). In the same study, the promoters of copper transporter genes CTR1 and CTR2 were identified to be repressible in the presence of copper sulphate (Xiong and Chen, 2020).

2.1.4. Inducible synthetic promoters

Fusion of either 4 tandem repeats of $UAS1_{XPR2}$ or three tandem repeats of UAS_{EYK1} to the minimal EYK1 promoter enhanced the promoter strength without affecting its inducibility by erythritol or erythrose (Trassaert et al., 2017). Tandem copies of the URS (Upstream regulatory sequence) of the fatty-acid inducible POX2 promoter were attached to the minimal TEF p/TEFinp to generate hybrid promoters that were 10-fold stronger than the native POX2p (Shabbir Hussain et al., 2017). Another inducible promoter was created by transferring the xylose-activator and the xylose operator sequence from *E. coli* to *Yarrowia lipolytica* (Wei et al., 2020). The *E. coli* XylO operator sequence was cloned upstream of TEF1 /LEU2 core promoter sequences. The strain also expresses the protein VPRHX generated by covalent fusion of *E. coli* XylR to endogenous transcriptional activation domains HSF and VPR. Xylose-inducible expression of the gene was observed in this strain. In addition, a xylose-inducible bidirectional promoter sequence was generated by sandwiching the xylose operator sequences between the Leu2p and TEF1p sequences. Expression of the reporters mCherry and GFP from the bidirectional promoter was observed only in the presence of xylose (Wei et al., 2020).

Using light as an inducer has several advantages over chemical inducers. Apart from being non-toxic, the light-induced transcriptional response can be immediate and reversible. Two light-inducible promoters have been constructed in *Yarrowia*. In one system, gene expression is prevented in the presence of green light and requires the presence of CarH-VPRH and CarO operator sequences in the promoter. CarH-VPRH consists of the light-sensitive bacterial transcription factor CarH fused to transcriptional activation domains VP64, p65, Rta and HSF1. CarH fusion protein can form tetramers only without a green light, binds to its cognate operator CarO sequences in the promoter, and activates transcription. The second optogenetic system is the blue light-induced system based on fusing the blue photosensitive protein EL222 to the transcriptional activator VP16 and C120 sequence fused to a minimal CYC_{102} promoter. The fusion protein binds to the C120 sequence only in the presence of blue light and activates transcription (Wang et al., 2022a, 2022b).

2.1.5. *Transcriptional terminators*

Terminators are key regulatory elements of gene expression and can hugely affect the half-life of mRNA. Apart from signalling the transcription end, they facilitate mRNA cleavage at the 3’end, followed by adding the 3’ poly A-tail. The absence of termination could result in an unstable mRNA that is unsuitable for translation. The *S. cerevisiae* cytochrome oxidase (ScCYC1) terminator sequence has been widely used in *Yarrowia lipolytica*. A series of synthetic terminator sequences were constructed in *S. cerevisiae* by tweaking its components, namely the efficiency element, the positioning element, the polyadenylation (poly(A) site), and T-rich regions around the poly(A) site (Curran et al., 2015). These terminators were also functional in *Yarrowia* and produced 60% more protein than the Sc *CYC1* terminator (Curran et al., 2015). Apart from ScCYC1, the endogenous XPR2 (full length and minimal) and LIP2 terminators have also been used (Nicaud et al., 2002). Unlike promoters, the research on transcriptional terminators has been relatively sparse in *Yarrowia lipolytica*.

2.2. *Transformation and genetic modification strategies*

Chen et al. (1997) optimized the key specifications for efficient one-step chemical transformation in *Y. lipolytica*. PEG (Polyethylene glycol)-4000 at 45% was optimal for transformation. Transformation was not successful with PEG-3350. It was found that 100 mM DTT (Dithiothreitol) and an incubation time of 60 min work optimally. Other reducing agents were not tested and combinations of DTT concentration and incubation time were tested and optimized. Additionally, cells grown for 16-20 h overnight on agar plates yield higher transformation (Chen et al., 1997). A more recent chemical transformation method employed a multi-step incubation strategy and improved the transformation efficiency further. The washed cells from plates were first incubated with 100 mM LiOAc (Lithium acetate), followed by the addition of carrier DNA and transformation DNA. Finally, PEG and more LiOAc were added and heat shock was performed at 39 °C (Leplat et al., 2015). Interestingly, the 1997 report by Chen et al. found that washing competent cells with distilled water before plating on selective plates leads to osmotic shock, which lowers transformation efficiency (Chen et al., 1997). A more recent protocol by Holkenbrink et al. reported resuspending the cells with water before plating (Holkenbrink et al., 2018). Hence, there are multiple approaches towards plating of cells after transformation.

Apart from LiOAc method, other transformation techniques have also been reported in *Y. lipolytica*. An agrobacterium-mediated transformation protocol was reported by Bredeweg et al. (Bredeweg et al., 2017), and a high-efficiency transformation protocol through electroporation was reported in 2018 by Markham et al. (Markham et al., 2018). A high-throughput chemical transformation method that can be performed in a 96-well plate format has also been reported (Leplat et al., 2015).

To improve the efficiency of homologous recombination, Jang et al. deleted KU70 and synchronized the cell cycle into S-phase by adding hydroxyurea. Thus, using a combination of biological and chemical approaches, they obtained a 90% success rate in constructing gene deletions (Jang et al., 2018). Holkenbrink et al. created an extensive CRISPR/Cas9-based toolbox for genetic modification in *Y. lipolytica*. More recently, another CRISPR/Cas9-based tool was developed, which does not require any homologous template or integration of selection marker and achieved gene integration in a single step (Holkenbrink et al., 2018). The toolbox allows both marker-free CRISPR/Cas9-assisted integrations and marker-based integrations (dominant markers with loxP/Cre options). They obtained >80% integration efficiencies using a non-replicating repair DNA fragment.

The efficiency of CRISPR-Cas9-mediated genome engineering in *Yarrowia lipolytica* is constrained by sgRNA expression and maturation, as well as the organism's strong reliance on NHEJ over HR for DSB repair. Expression of sgRNAs *via* synthetic Pol III-tRNA hybrid promoters ensured that they are correctly processed enabling efficient genome editing. Schwartz et al. deployed SpCas9 for effecting a markerless gene disruption and integration (Schwartz et al., 2016). They obtained ≥92% single-gene disruption using Pol III-tRNA hybrid promoter SCR1′-tRNAGly and > 64% markerless HR with a donor. Schwartz et al. then screened genomic loci for growth neutrality and efficient expression and identified five integration sites (AXP, XPR2, A08, D17, MFE1), with consistent GFP expression. They validated their strategy by integrating four genes for a semi-synthetic lycopene pathway at multiple loci (Schwartz et al., 2017b).

Abdel-Mawgoud and Stephanopoulos (2020) also demonstrated that direct tRNA-sgRNA fusions improve editing robustness, while the presence of extra “intergenic” nucleotides can perturb sgRNA structure and reduce activity (Abdel-Mawgoud and Stephanopoulos, 2020). Schwartz et al. implemented CRISPR interference in Yarrowia to enhance HR by repressing NHEJ factors. Using multiplexed sgRNAs (targeting KU70/KU80) and enhancing repression with dCas9-Mxi1, they achieved HR rates up to ~90% without deleting NHEJ genes (Schwartz et al., 2017a, 2017b).

Schwartz et al. converted their dCas9 system into CRISPRa by swapping a repressor for the VPR activator (VP64 + p65 + Rta) and expressing sgRNAs from a SCR1′-tRNAgly hybrid Pol III promoter. They used CRISPRa to activate transcriptionally silent native β-glucosidases (BGL1/BGL2), enabling growth on cellobiose. Bae et al. demonstrated Target-AID (Activation induced cytidine deaminase) base editing in *Yarrowia* by expressing nCas9(D10A) fused to Cytidine deaminase and N-uracil glycosylase inhibitor along with guide RNAs from a single plasmid, enabling mutations within the target locus (Bae et al., 2020). Besides Cas9, the Cas12 has also been used for editing *Yarrowia lipolytica*. Yang et al. used CRISPR-Cas12a (AsCpf1) editing to delete the CAN1 gene with efficiencies of >72% (Yang et al., 2020).

In an interesting approach to assembling multiple pathway genes into a single cell, Guo et al. constructed a plasmid with the essential replication and segregation elements of *Y. lipolytica* chromosomes. The genes to be assembled were constructed through PCR, having overlapping ends. These PCR products were transformed along with the linearized plasmid for direct *in vivo* assembly using the homologous recombination of *Y. lipolytica*. A 90% yield was observed for this approach (Guo et al., 2020). However, this artificial chromosome is episomal. Cas9-hBrex27 fusion protein has been shown to improve integration efficiency for multifragmenting pathway assemblies. hBrex27 is the exon 27 of the Human BRCA2 (hBRCA2) gene (Jiang et al., 2025). Even though the molecular biology and genetic engineering tools are not as extensive as yet for *Yarrowia lipolytica* as we see for other yeasts like *Saccharomyces cerevisiae*, there have been considerable efforts to achieve that. This is exemplified by the many techniques and tools devised to date and reviewed above.

2.3. Adaptive laboratory evolution

Adaptive laboratory evolution (ALE) is a powerful tool in biotechnology as it can improve microorganism's stress tolerance and substrate utilization capabilities (Fig. 2). ALE has been used to enhance the production of lipids (Liu et al., 2015a, 2015b; Sofeo et al., 2024) and natural products like limonene (Li et al., 2021; Yang et al., 2017) in *Yarrowia lipolytica* (Table 2). It has also been used to improve tolerance of *Y. lipolytica* towards various stress like ferulic acid, vanillic acid, glycerol, fatty acid distillate and ionic liquids (Table 2) (Coleman et al., 2023; Sha et al., 2023; Sofeo et al., 2024; Tsirigka et al., 2023; Walker et al., 2019; Wang et al., 2021). In ALE, the strains are subjected to increasingly higher stress concentrations, which leads to the accumulation of beneficial mutations favoring growth under stressful conditions. Once adapted, whole genome sequencing (Tsirigka et al., 2023) and transcriptomics (Li et al., 2021; Sofeo et al., 2024; Tsirigka et al., 2023) analysis is performed to identify the genetic changes associated with the adaptation to stress or improved production and performance. Another application of ALE is to counter the effect of product toxicity. Limonene is toxic to *Y. lipolytica*, a major obstacle in achieving high titer. Strain with tolerance towards higher concentrations of limonene was obtained through ALE. Transcriptomics was used to identify 82 genes highly expressed in tolerant strains. Out of these, 8 genes, when overexpressed in wild-type strain, led to higher tolerance towards limonene. Specifically, overexpressing the protein YALI0F19492p led to an 8-fold increase in the product titer (Li et al., 2021). To improve the rate of random beneficial mutagenesis for ALE, strains are also subjected to EMS mutagenesis to introduce these mutations, possibly leading to improved performance (Liu et al., 2015a; Tsirigka et al., 2023).

Recently, ALE was also conducted *via* anaerobic fermentation of *Y. lipolytica* ACA DC 50109 to enhance its inhibitory effect towards hexanoic acid, the longest chain in the SCFA pool, which is widely known to inhibit cell growth during the fermentation process. At the end of 27 adaptation rounds (~135 generations), cells could maintain the growth under the stress of hexanoic acid concentration up to 6.24 g/L, where this condition would not be achieved when wild type strain is used. Transcriptomic results justified that the evolved strain possessed a high expression of the FAT1 gene, which is important for mobilizing long-chain acids within the cells (Morales-Palomo et al., 2024).

Generally, ALE has been used to adapt *Y. lipolytica* strains to different media with varying carbon sources, like glycerol (Tsirigka et al., 2023), lignocellulosic material (Sha et al., 2023; Wang et al., 2021), or lipid-rich side stream (Sofeo et al., 2024), and also higher concentrations of glucose (Yang et al., 2017). Other non-standard industrial waste streams used for ALE on *Y. lipolytica* are aqueous side streams from hydrothermal liquefaction (Coleman et al., 2023) or organic solvents such as ionic liquids (Walker et al., 2019). Population enrichment coupled with ALE has also been performed to isolate superior lipid producers in the cell population (Daskalaki et al., 2019; Liu et al., 2015a). The common methods employed for cell enrichment include floating selection, Nile red screening, *etc.*

2.4. Strain mating

Strain mating is a versatile genetic engineering tool where new strains generated derive one set of chromosomes from two different parents. *Yarrowia lipolytica* has a single locus for mating type in its genome, harbouring either a *MAT*A or a *MAT*B allele with a low mating efficiency. The MATA locus gene has been cloned and was found to encode two proteins, *MAT*A1 and *MAT*A2. *MAT*A1 and the intergenic region between *MAT*A1 and *MAT*A2 are required to induce sporulation, while *MAT*A2 is necessary to repress conjugation in *MAT*B strains (Kurischko et al., 1999). A mating type switch has been reported by replacing *MAT*A with *MAT*B cassette through two steps of MAT locus deletion plus replacement strategy. The subsequent mating efficiency of the resulting strain was low (Rosas-Quijano et al., 2008). A recent study also attempted to use site-specific homologous recombination to convert haploid *MAT*A type to *MAT*B scarlessly in a single transformation step.

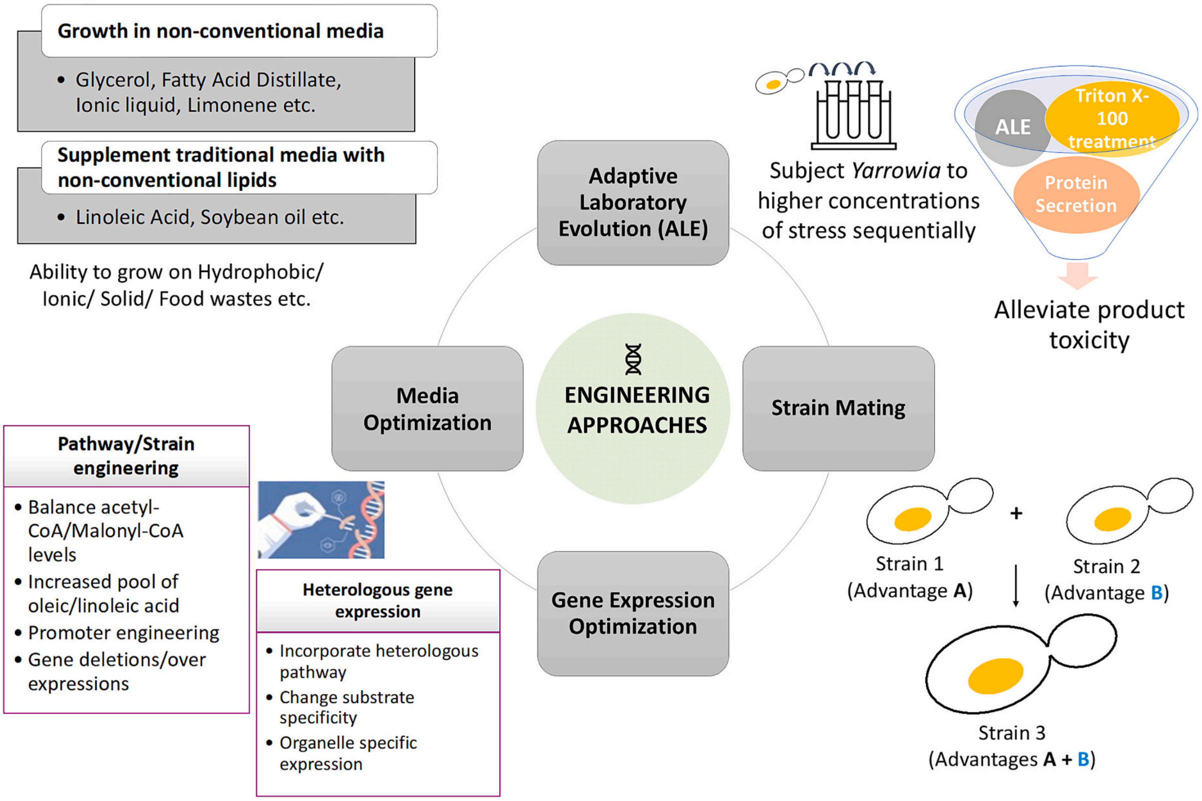


Fig. 2. Summary of biotechnological approaches taken for *Yarrowia lipolytica.*

Table 2
Use of adaptive laboratory evolution for biotechnological applications in *Yarrowia lipolytica*.

Product	Engineering Strategy	Outcome	Time (days)	Ref.
Limonene	ALE with overexpression of 8 candidate genes identified from transcriptomics	8 fold improvement in YALI0F19492p overexpressing stain	5	(Li et al., 2021)
Lipid	EMS mutagenesis, Enrichment by floating selection, nile red screening	39.1 g/L	6	(Liu et al., 2015a)
Lipid	Population enrichment in cells that accumulate intracellular lipids; carbon starvation immediately followed a lipid accumulation phase	30% improvement in lipid content	11	(Daskalaki et al., 2019)
Lipid	Passaging in higher concentrations of lipid rich cocoa fatty acid distillate (CFAD) containing media	4.8 g/L	1	(Sofeo et al., 2024)
Ferulic Acid tolerance	ALE to improve tolerance for up to 1.5 g/L Ferulic acid	1.5 g/L	2	(Wang et al., 2021)
Lipid	ALE to improve tolerance to vanillic acid up to 1.5 g/L	7.4 g/L	5	(Sha et al., 2023)
([EMIM][OAc]) tolerance	ALE to improve tolerance to ionic liquids up to 18%	Improved IL tolerance up to 18%	2	(Walker et al., 2019)
Succinic acid	ALE performed in increasingly higher concentrations of glucose (Up to 120 g/L glucose)	65.7 g/L	4	(Yang et al., 2017)
TAL (triacetic acid lactone)	ALE performed in increasingly higher concentrations of HTL-AP side stream (Hydrothermal liquefaction-Aqueous phase up to 15%)	1.2–1.3 g/L	7	(Coleman et al., 2023)
Lipid	EMS mutagenesis followed by ALE in higher concentration of glycerol (up to 20%)	>0.5 g/L	3	(Tsirigka et al., 2023)

The mating-type switched strain was then mated with another *MATA* strain at a low temperature and the addition of sodium citrate to generate a *MATA*/*MATB* diploid strain (Han et al., 2021). Several factors have been identified to be important for sporulation and mating in *Y. lipolytica*. Mitogen-activated protein kinase (MAPK) (Cervantes-Chávez and Ruiz-Herrera, 2006) and cyclic-AMP dependent protein kinase (PKA) (Cervantes-Chávez et al., 2009) pathways have been shown to play a role in dimorphism and mating. Deleting the *tpk* gene in the PKA pathway and the ste11 gene in the MAPK pathway resulted in lower mating efficiency, while the double mutant was incapable of mating (Cervantes-Chávez et al., 2009). Transcription factors like YlRim101p have also been shown to be important for mating and sporulation in *Y. lipolytica* (Lambert et al., 1997). The strain mating approach combined two haploid strains, one capable of utilizing xylose efficiently and the other capable of overproducing metabolites to generate novel diploid xylose-utilizing strains. In this study, three xylose-utilizing strains were generated, which overproduced α-linolenic acid (0.52 g/), riboflavin (96.6 mg/L), and triacetic acid lactone (2.9 g/L) (Fig. 2) (Li and Alper, 2020). A slightly different approach of protoplast fusion has also been used for cells of the same mating type in *Y. lipolytica* to generate prototrophic diploids. This study confirmed genetic recombination through a fusion of protoplasts. This technique could also be used to create strains with higher productivity from low-producer parent strains (Shah et al., 1989).

3. Bioremediation application *via* degradative pathways

The manufacturing process in petrochemical and petroleum industries usually generates a substantial volume of waste and byproducts, including heavy metals, hydrocarbons, fats, and petroleum fractions, which will negatively affect the environment and overall ecosystem if not treated properly(Johnson and Affam, 2019). Degradation of these waste materials with microorganisms *via* bioremediation could be a promising alternative to solve this issue.

Fortunately, *Y. lipolytica* has been regularly isolated from environments heavily polluted by hydrocarbons, which has also prompted interest in its use in the bioremediation of oil-contaminated soils and waters. Generally, microorganisms face challenges in efficiently assimilating hydrocarbons due to the compounds' low solubility in water. To overcome this limitation, *Y. lipolytica* specifically produces biosurfactants, which lower surface tension to facilitate the formation of stable emulsions (Chrzanowski et al., 2008). This yeast has previously demonstrated the capability to utilize pure aliphatic and aromatic hydrocarbons as a carbon source without the need for additional chemical surfactants. The findings indicate that yeast growth and degradation efficiency are influenced by various factors, including strain characteristics, the type and chain length of hydrocarbons, and the prevailing culture conditions (Hassanshahian et al., 2012; Margesin et al., 2003).

Several *Yarrowia sp.* strains, especially aquatic ones, were previously reported to assimilate n-hexadecane, one of the components in crude and diesel oil. Their degradation rates were described to range between 39% and 78%, with a maximum incubation period of up to 7 days. *Y. lipolytica* CMGB32 was utilized to degrade *n*-hexadecane by producing biosurfactants with a high potential for bioremediation. The produced biosurfactants could serve as a prosperous platform to eliminate heavy and toxic metals (*i.e.* Cd and Pb) and resist emerging pathogens coming from wastewater (Csutak et al., 2024).

Another long-chain alkene, pentadecane and heptadecane, was also described to be used in the growth media of *Y. lipolytica* CCY 30-26-36 to accumulate 12-18% (*w*/w) intracellular lipid, which mainly consisted of palmitic, palmitoleic, linoleic, and oleic acid (Matatkova et al., 2017).

In addition to its potential to consume pure alkanes, the *Y. lipolytica* strains obtained from marine and terrestrial contaminated settings also exhibit the ability to degrade complex mixtures of hydrocarbons, such as crude and diesel oil. The high cell surface hydrophobicity and emulsifying activity in these cultures are crucial in facilitating the biodegradation process (Hassanshahian et al., 2012).

The shorter-chain length n-alkanes (C9-C16) present in crude oil undergo easier degradation compared to their longer-chain counterparts (C17-C25). This occurs because longer-chain hydrocarbons appear in a solid form at room temperature, making it challenging for yeast to solubilize and assimilate them (Iheanacho et al., 2014). Studies have demonstrated that enhancing the initial inoculum or cultivating yeast at high cell densities are effective strategies for improving crude oil degradation without the need of adding additional nutrients (Ferreira et al., 2012). Concentrations of crude oil below 2% (*v*/v) do not inhibit *Y. lipolytica* growth and hydrocarbon degradation. However, inhibition effects are observed with concentrations exceeding 4% (v/v) due to the toxic impact of high hydrocarbon and phenolic compound levels (Ferreira et al., 2012; Lopes et al., 2022).

The degradation of hydrocarbons by *Y. lipolytica* is predominantly considered as an aerobic process. The assimilation occurs through the β-oxidation pathway, which demands high levels of oxygen. Increased agitation rates have been shown to enhance crude oil degradation significantly, but no degradation occurs under partly anaerobic conditions (Ferreira et al., 2012; Lopes et al., 2022).

Other than polluted hydrocarbons, heavy metal contamination has also become a crucial issue in the environment caused by the petroleum and petrochemical industries. Many studies have been conducted to investigate the interactions of metals with different types of fungal strains, including *Yarrowia lipolytica*. Various types of this strain possess distinct characteristic features that enable them to withstand the stress caused by the existence of different metals.

Previously, two marine *Y. lipolytica* strains (NCIM 3589 and NCIM 3590) were found to exhibit a high tolerance towards heavy metals, such as (V), Co (II), Cr (III), Cu (II), Ni (II), Pb (II), and Zn (II); justifying that this cell line could be an excellent candidate for removing metal ions from contaminated wastewater in the long term (Bankar et al., 2018). Growth under the stress from these metals was also investigated on the sterile glass surface, and morphological differences were observed in the biofilm surface for the cells grown under the metal stress compared with the control (Bankar et al., 2018). These findings remark a significant contribution in the use of *Y. lipolytica* in bioremediation applications for the recovery of heavy metals from contaminated environments.

Bioremediation of copper from wastewater was also previously reported using *Y. lipolytica* AUMC 9256, and it was shown that the inhibitory effect from this metal towards the cells was reported as high as 1.9 g/L (El-Sayed, 2018). However, they justified that different strains of *Y. lipolytica* might exhibit different tolerance towards metal ions. This occurred in attribution to the formation of cysteine-rich metallothioneins and melanin as metal binding molecules, production of H^+ ATPase and superoxide dismutase, as well as sequestration on the plasma membrane and cell wall.

Recently, the deletion of MET25 and YALI0F14047g genes within this cell line's sulphur metabolic pathway was also successfully reported to provide sulfide at 550 ppm without disturbing cell growth (Yang et al., 2024b). This sulfide-producing strain was then subsequently utilized to remove heavy metals such as Cd, Cu, and Pb with over 90% removal efficiency.

4. Single cell protein, single cell oil, enzyme production, and use of non-conventional feedstock

Single cell protein (SCP), also known as microbial protein or protein biomass, can be used as an alternative protein source in a world where natural protein is deficient due to the ever-growing population. SCPs are dried biomasses that can be used as supplements in foods and animal feeds due to their high protein content (Jach et al., 2017). SCPs need to be utilized as they contain a higher content of essential amino acids, such as lysine and methionine, which are lacking in animal and plant sources (Jach et al., 2017; Suman et al., 2015). *Yarrowia lipolytica* has been deemed a very good candidate for producing high levels of protein content due to its unique ability to metabolize oils and hydrophobic substrates, aided by extracellular lipases and emulsifiers (Spalvins et al., 2020).

A wide variety of waste substrates has been studied that are able to produce high amounts of protein. Substrates such as vegetable oil waste, agricultural waste, fish or seafood wastes, and crude glycerol have been known to generate various compounds, including SCPs (Gottardi et al., 2021). Table 3 shows that SCPs from *Y. lipolytica* could be used in different type of applications. For instance, it effectively utilizes waste cooking oil (WCO) in fermentations without requiring prior emulsification, significantly reducing production costs. Optimal SCP production occurred with a medium containing 27.5 g/L WCO and a C/N ratio of 5-10, resulting in a high *Y. lipolytica* biomass concentration of 57.37 g/L and moderate protein yield of 12.6% (Spalvins et al., 2020).

One study has reported that using sugarcane molasses, a type of agricultural waste, was found to be better than common carbon-rich substrates like WCO and crude glycerol. These substrates undergo acid-enzymatic hydrolysis, where their fermentable monosaccharides are utilized by *Y. lipolytica* (Drzymała et al., 2020). Using an engineered strain, the SCP yield was found to be at 151.2 g/L after 100 h in a 10 L bioreactor fermentation scale (Yan et al., 2018). The strategy involved

Table 3
Production of Single Cell Proteins in *Yarrowia lipolytica.*

Waste Substrate	Yarrowia Strain	Biomass (g/L)	Protein Content	Fat Content (%)	Application	Time (h)	Ref.
Biofuel production waste	A-101	N.A	40% to 50%	N.A	Human nutritional supplement	12	(Jach et al., 2017)
Oat bran	A-101	9.35	45% DCW	20% DCW	N.A	120	(Drzymała et al., 2020)
Waste cooking oil	WT	57.37	57.37 g/L	N.A	Animal feeds	120	(Spalvins et al., 2020)
Waste cooking oil	YLY (URA3 replace with SUC2)	17.9	17.9 g/L	N.A	Fish feeds	89	(Yan et al., 2018)
Raw glycerol	YLY (URA3 replace with SUC2)	22.3	22.3 g/L	N.A	Fish feeds	89	(Yan et al., 2018)
Sugarcane Molasses	YLY (URA3 replace with SUC2)	151.2	151.2 g/L	N.A	Fish feeds	89	(Yan et al., 2018)
Glycerol	MUCL 28849	31	31 g/L	20%	N.A	60	(Fontanille et al., 2012)
Tuna head protein hydrolysate	VTCC 0544	5.92	64%	9.60%	Tuna processing	72	(Ta et al., 2024)
Glycerol with modified FM22	WT + pINA1297	170.3	170.3 g/L DCW	N.A	N.A	136	(Pan et al., 2023)
Mutton Fat	CICC1778	14.1	14.1 g/L	32.60%	Cocoa Butter Equivalent lipid	72	(Xiong et al., 2015)
Solid cocoa fatty acid distillates	CBS 2070	>40	> 40 g/L DCW	>25%	N.A	48	(Peterson et al., 2023)
Glycerol	N.A	N.A	470 g/kg	N.A	Feed additives in milk	48	(Stefańska et al., 2018)
Crude glycerol	MUCL 28849	15	15 g/L	5.52% DCW	Meat nutrition quality	24	(Dedousi et al., 2023)
N.A	N6, D1	N.A	N·D	N.A	Dietary supplement	24	(Licona-Jain et al., 2020)
N.A	WT	N.A	N·D	N.A	Growth and antioxidant capacity	48	(Fei et al., 2022)
Glycerol from rapeseed biofuel	A-101.	96.5%	43.50%	1.50%	Animal feeds		(Czech et al., 2016)
Cassava peel	QU69	N.A	34%	N.A	Food additive for Immunomodulatory effects	216	(Neuls et al., 2021)

N.A: Information not available.

disrupting the URA3 gene (involved in uracil biosynthesis) in *Y. lipolytica* using homologous recombination, replacing it with an SUC2 expression cassette. This disruption rendered the strain auxotrophic for uracil, allowing for selection on 5-FOA-containing media. This enhanced overexpression due to the redirected metabolic resources towards SCP and lipase production. This process demonstrates a targeted genetic modification approach to engineering this strain for specific metabolic goals (Yan et al., 2018).

The SCPs of this cell line act as an alternative protein source substitute for animal feed that can benefit in terms of growth performance and better health benefits. For example, a study was conducted on shrimp feed *via* feeding trials and biochemical analysis. Results showed that the optimal cell inclusion for Pacific white shrimp was 2.68% of the diet, which was equivalent to 10.54% of the fishmeal (FM) content. The biomass inclusion led to reduced serum triglyceride and total cholesterol levels, highlighting its beneficial impact on lipid metabolism. Additionally, *Y. lipolytica* inclusion enhanced antioxidant capacity, demonstrated by increased total antioxidant capacity and reduced malondialdehyde content in shrimp diets (Zhang et al., 2024).

Other than generating SCPs, this particular cell line is also known for its ability to generate substantial quantities of other enzymes and other by-products, such as lipases (Table 4). Lipases are enzymes that facilitate the breakdown of triglycerides into free fatty acids and glycerol in the presence of water (Carvalho et al., 2023). Lipases are manufactured during solid-state fermentation (SSF), which refers to the fermentation of solid substrates in the absence of free water yet with sufficient moisture to sustain microbial growth and metabolism (Imandi et al., 2013). Residues from oleaginous cultures, such as soybean and olive, are favoured due to their existing lipid content, which induces lipase production (Do Nascimento et al., 2022). For example, the fermentation medium combining a 50:50 ratio of andiroba oil cake and soybean meal demonstrated high effectiveness in lipase production, yielding maximum lipolytic activity of 82.52 U/g with the addition of 0.001% Tween 80 and 1.5% soybean oil. LIP2 proteins were identified by electrophoresis, and applying this lipase to oil hydrolysis achieved up to 63% hydrolysis in 24 h. This study showed the potential of using Amazon region by-products with soybean meal to produce lipase for effective fish oil hydrolysis, facilitating polyunsaturated fatty acid generation (Carvalho et al., 2023).

Lipase regulation primarily responds to various external stimuli. For instance, the regulation of LIP2 expression, which encodes the primary extracellular lipase (Lip2p), involves cellular responses to glucose, which represses lipase production and triacylglycerol molecules. This activates specific mechanisms like specific-for-oleic acid (SOA) genes for free fatty acid utilization. Additionally, complex nitrogen sources such as peptone and tryptone appear to enhance lipase production (Do Nascimento et al., 2021). Lipase production rises with higher relative percentages or increased concentrations of C18:n fatty acid esters in the substrate. Both the synthesis and secretion of lipase (both intra- and extracellular) increase with rising lipid concentrations, although excessively high levels in the growth medium can be cytotoxic (Do Nascimento et al., 2022).

5. High-value biochemical production

5.1. TCA cycle

The yeast *Yarrowia lipolytica* secretes large amounts of a broad spectrum of organic acids, including the tricarboxylic-acid (TCA) cycle intermediates citric acid (CA), isocitric acid (ICA), and 2-ketoglutaric, as well as pyruvic acids (Table 5).

5.1.1. Citric acid

The global market of citric acid (CA) stands as one of the tremendous and rapidly expanding markets in the biotechnological industry. With its global production of 1-1.5 million tons (Berovic and Legisa, 2007), CA has become an interest in many industrial applications: pharmaceuticals, confectionery, metallurgy, flavours, oil stabilisers, cosmetics, and perfumery. Thus far, filamentous fungi *Aspergillus niger* has been commercially used to produce CA *via* submerged or solid-state fermentation by using molasses-based media as the sole carbon source. Despite this success, the downstream processing still remains challenging due to the complex purification process, which involves product precipitation in the form of calcium salt, followed by specific treatment with sulfuric acid. As a result, the overall quantity of waste generated is similar to the amount of CA produced (Kamzolova and Morgunov, 2017). *Yarrowia lipolytica*, on the other hand, is well known for its ability to produce CA from various substrates in high titer and yield. However, the production of CA and its isomer, isocitric acid (ICA), occurs simultaneously *via* the TCA cycle (Fig. 3). Further metabolic engineering work is therefore required to control and enhance the targeted production of *CA*. Previously, recombinant *Y. lipolytica* strain overexpressing isocitrate lyase (ICL1) was successfully reported to improve the proportion of CA from 88 to 90% to 94-99% using glucose or glycerol as a carbon source (Förster et al., 2007).

5.1.2. Isocitric acid

Isocitric acid (ICA) is a structural isomer from citric acid, one of the main products in the TCA cycle (Fig. 3). It has been widely used as a precursor to obtain active pharmaceutical ingredients (API), surfactants, and biologically-produced food additives. Production of ICA *via* biological approaches is still preferable since the resulting product contains only natural isomers (Bullin et al., 2019; Fickers et al., 2020; Giannis and Mousavizadeh, 2019; Kamzolova et al., 2020; Kamzolova and Morgunov, 2019). Wild-type *Yarrowia lipolytica* generally produces 8-16% ICA during growth, using glycerol or carbohydrates as carbon sources (Förster et al., 2007). Optimizing the metabolic pathway is therefore required to enhance the yield and titer of ICA further. Holz et al. (2009) previously altered the ICA proportion up to 66-71% *via* overexpression of the aconitase (ACO1) gene in the *Y. lipolytica* H222 strain (Holz et al., 2009). Moreover, overexpression of mitochondrial citrate synthases (CIT1) was also previously reported to enhance the titer of ICA up to 22.97 g/L with a yield of 0.15 g/g (Hapeta et al., 2020). The use of ester-aldehyde fraction (EAF) as a substrate for the production of isocitric acid was also investigated by Kamzolova et al. (2021). It was found out that *Y. lipolytica* VKM Y-2373 is able to convert ethanol waste to isocitric

Table 4
Enzyme Production in *Yarrowia lipolytica.*

Waste Substrate	Product	Yarrowia Strain	Culture Conditions	Titer, Yield	Time (h)	Ref.
Waste cooking oil	Lipase	YLY (URA3 replace with SUC2)	28 °C, pH 6, 300-600 rpm	980 U/mL	89	(Yan et al., 2018)
Raw glycerol	Lipase	YLY (URA3 replace with SUC2)	28 °C, pH 6, 300-600 rpm	1028 U/mL	89	(Yan et al., 2018)
Sugarcane Molasses	Lipase	YLY (URA3 replace with SUC2)	28 °C, pH 6, 300-600 rpm	16,420 U/mL	89	(Yan et al., 2018)
Andiroba oil cake and soybean meal	Lipase	IMUFRJ50682	SSF, 28 °C	82.52 U/g	72	(Carvalho et al., 2023)
Mustard oil cake	Lipase	NCIM 3589	SSF, 30 °C	57.89 U/g	96	(Carvalho et al., 2023)
Two-phase olive mill waste	Lipase	IMUFRJ 50682	SSF, 28 °C	486 U/g	96	(Lopes et al., 2016)
Canola meal	Protease	CDBB-L-232	SSF, 30 °C, pH 7	188.75 U/L	48	(López-Trujillo et al., 2023)
N.A	Lipase	YLL2	28 °C	4125 U/mg	84	(Chen et al., 2021)

N.A: Information not available; SSF: Solid State Fermentation.

Table 5
Bioproducts derived from the TCA cycle.

Product	Substrate	Engineering Strategy	Titer (g/L)	Yield (g/g substrate)	Scale (Working Volume)	Time (h)	Ref.
Isocitric acid	Glucose	CIT1↑ or CIT2↑	22.97	0.15	Bioreactor (2 L)	72	(Hapeta et al., 2020)
Isocitric acid	Ethanol industry waste	NA	65	0.65	Bioreactor (5 L)	96	(Kamzolova et al., 2021)
Itaconic acid	Glucose	AtCAD↑, ACO↑, AMPD↓	4.6	0.058	Bioreactor (1.5 L)	168	(Blazeck et al., 2015)
Itaconic acid	Glucose	AtCAD↑, MTT↑	22.02	0.056	Fed-Batch Bioreactor (3.5 L)	200	(Zhao et al., 2019)
Itaconic acid	WCO	AtCAD-ePTS1↑, POT1↑, ΔICL	54.55	0.3	Bioreactor (3 L)	144	(Rong et al., 2022)
Itaconic acid	Glucose	ΔICL1,ΔIDP, ΔDGA1, ΔDGA2, ΔLRO1, ΔARE1, MTT↑, AtCAD↑, MDT↑	94.8	0.26	Bioreactor (50 L)	408	(Fu et al., 2024)
Succinic acid	Crude glycerol	ΔSDH5	160	0.4	Fed-Batch Bioreactor (1 L)	400	(Gao et al., 2016)
Succinic acid	Glycerol	ΔSDH5, ΔACH1,ScPCK↑, SCS2↑	110.7	0.53	Fed-Batch Bioreactor (2.5 L)	138	(Cui et al., 2017)
Succinic acid	Fruit & vegetable waste supplemented with corn steep liquor	ΔSDH5	140.6	0.47	Fed-Batch Bioreactor (1 L)	324	(Li et al., 2018)
Succinic acid	Glucose & corn steep powder	ΔSDH5, ΔACH1,PYC↑, TbFrd↑, EcFUM↑, MDH1↑	111.9	0.79	Fed-Batch Bioreactor (5 L)	62	(Cui et al., 2023)
Succinic acid	Corncob hydrolysate	ΔSDH5, ΔACH1,PYC↑, TbFrd↑, EcFUM↑, MDH1↑, YlGsh2↑	45.34	0.71	Fed-Batch Bioreactor (5 L)	32	(Zhong et al., 2024)
α-KG	Glucose	PDA 1↑	43.3	3.7	Bioreactor (1.5 L)	144	(Guo et al., 2014)
α-KG	Glycerol	MmACL↑, ACS1↑	56.5	0.565	Bioreactor (1.5 L)	144	(Zhou et al., 2012)
α-KG	Glycerol	PYC1↑, IDP1↑	186	0.36	Bioreactor (0.6 L)	117	(Yovkova et al., 2014)

acid, giving 65 g/L product titer after 4 days of fermentation (Kamzolova et al., 2021).

5.1.3. Succinic acid

Succinic acid (SA) is an essential precursor for the production of high-value chemicals, such as tetrahydrofuran, 1,4-butanediol, polybutylene succinate (PBS), and γ-butyrolactone (Choi et al., 2015; Yang et al., 2017) Due to its broad applications, the SA market is projected to hit an annual growth rate of 8.2%, with an anticipated market value surpassing USD 200 million by 2026 (Kim et al., 2023). This molecule is also classified as 1 of the top 12 potential value-added bio-based chemicals by the US Department of Energy (Werpy and Petersen, 2004). Currently, SA is predominantly manufactured *via* chemical catalysis using maleic anhydride as the starting material. However, sustainable bioproduction of SA from renewable resources is highly preferred to supplement and substitute those of produced chemically for environmental concerns (Ko et al., 2020; Raj et al., 2022). SA serves as a native intermediate in the TCA cycle of many microorganisms and could be generated through both reductive and oxidative branches. The market of bio-based SA was estimated to reach US$ 14.1 billion (Gao et al., 2016), justifying the importance of producing this molecule *via* biotechnological approaches.

Y. lipolytica shows an innate capability to grow in acidic conditions, thus becoming one of the most suitable host candidates to accumulate SA production. Gao et al. (2016) successfully produced 160 g/L SA from crude glycerol with a yield of 0.4 g/g by knocking out the sdh5 gene encoding succinate dehydrogenase (Gao et al., 2016). SDH is generally responsible for the conversion of SA to fumaric acid, and SDH5 is a subunit of SDH, which is crucial for SDH stability and activity. Thus, deleting this gene will cause the SA accumulation and secretion out from the cells. Further deletion of CoA-transferase gene encoding *Ylach* with simultaneous corporation of phosphoenolpyruvate carboxykinase from *Saccharomyces cerevisiae* (ScPCK) and endogenous succinyl-CoA synthase beta subunit (SCS2) was also proven to significantly reduce the formation of acetic acid byproduct and enhance titer of SA, respectively (Cui et al., 2017). The best result is the SA product titer of 110.7 g/L (53% yield) was achieved *via* fed-batch fermentation without pH control.

Cui's group also successfully introduced a novel reductive TCA cycle for NADH regeneration to improve the productivity of SA. This was done by co-expressing fumarate reductase from *Trypanosoma brucei* (TbFrd), phosphoenolpyruvate carboxykinase from *Saccharomyces cerevisiae* (ScPCK), and endogenous malate dehydrogenase (Mdh1), followed by further adaptive laboratory evolution strategy (Cui et al., 2023). As their best results, 111.9 g/L SA with a yield of 0.79 g/g was successfully obtained.

Recently, it was reported that furfural became one of the inhibitors for the growth of *Y. lipolytica* in lignocellulosic biomass. Fortunately, the same group found that the incorporation of glutathione synthetase encoding YlGsh2 could solve this issue. As the best result, the engineered strain successfully produced 45.34 of SA (0.71 g/g yield) after 32 h of fed-batch fermentation using corncob hydrolysate in 5 L bioreactor (Zhong et al., 2024).

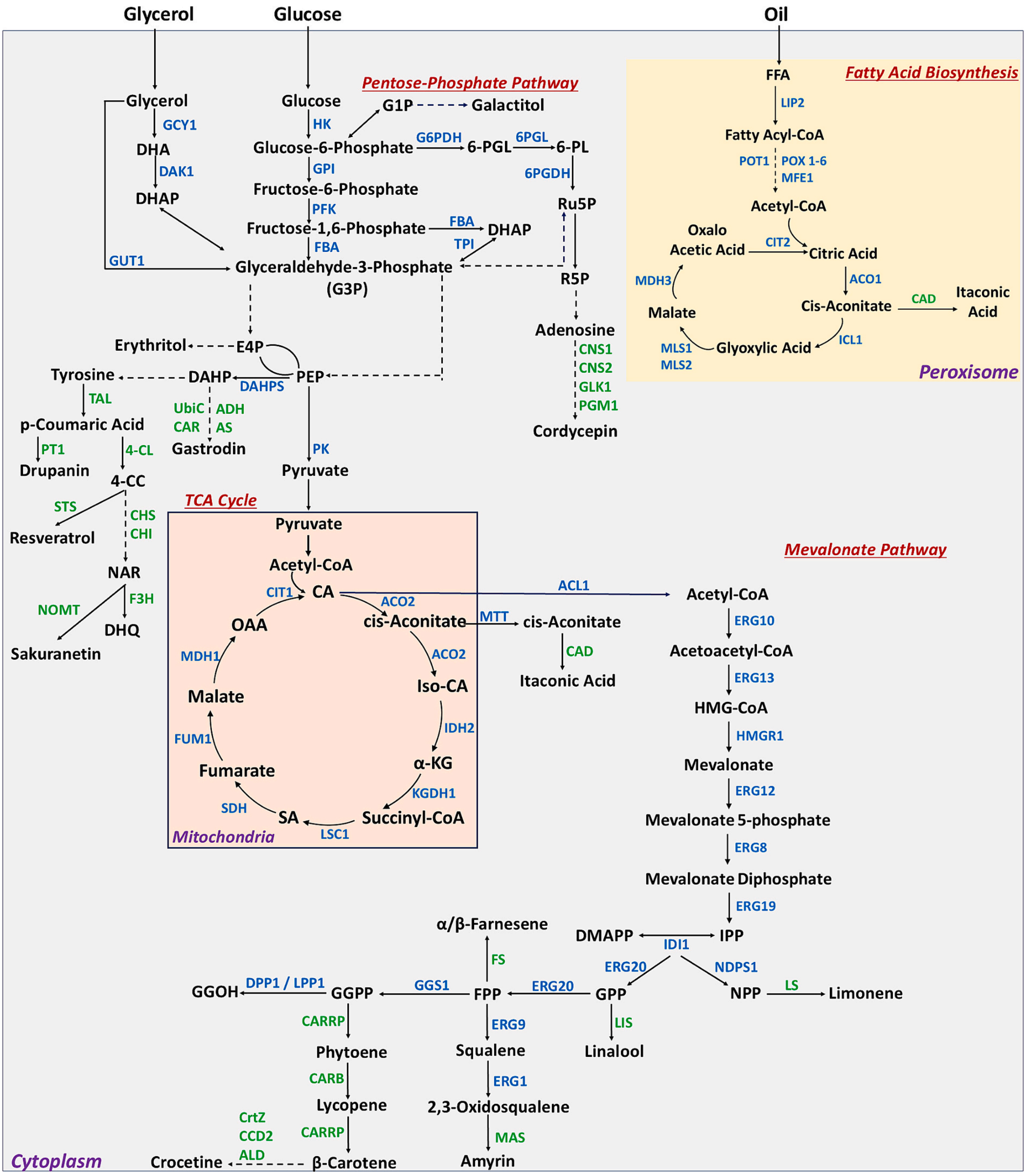
5.1.4. Itaconic acid

Itaconic acid is an organic acid which is naturally produced in several Aspergillus species and has also been recognized by the US Department of Energy as one of the top 12 value-added chemicals from biomass (Werpy and Petersen, 2004). The potential market of IA is expected to hit $260 million by 2025 due to its significance as a substitute for petrochemical-based molecules (Nuss and Gardner, 2012; Sriariyanun et al., 2019). IA has also been extensively used as a co-monomer for the production of coatings, thickeners, synthetic fibres, and coatings in attribution to its excellent properties (Willke and Vorlop, 2001; Zhao et al., 2018).

Aspergillus terreus has been utilized for the industrial production of IA, giving a high titer of >160 g/L of product (Kuenz et al., 2012). The fermentation process, however, still suffers from poor growth in media optimal for itaconic acid production. Fermentation with *Ustilago maydis* (Geiser et al., 2016) and *Ustilago cynodontis* (Hosseinpour Tehrani et al., 2019) have also been reported to produce IA up to 220 g/L naturally. Despite this success, submerged fermentation of filamentous fungi in a bioreactor remains challenging. The highly branched mycelial filaments contribute to the elevation of broth viscosity during fermentation, resulting in poor mixing and aeration in the bioreactor (Kubicek et al., 2011; Porro and Branduardi, 2017). Additionally, fermentation of most filamentous fungi requires the addition of alkali to maintain a neutral pH condition, which increases the probability of bacterial contamination (Cui et al., 2017; Li et al., 2021).

Y. lipolytica shows promising capabilities as an attractive host in attribution to its high tolerance towards environmental stresses, including its robust growth in low pH, low temperatures, and high salt concentrations (Gonçalves et al., 2014). Its immense tolerance in acidic conditions allows the accumulation of IA within the cells (Zhao et al., 2019). Secondly, this oleaginous yeast displays versatility in utilizing various carbon sources for its growth, such as waste cooking oil (WCO). This enables waste valorization of side streams, thus making the whole process more cost-efficient (Li et al., 2022; Pang et al., 2019). Thirdly, *Y. lipolytica* exhibits multiple pathways for generating and accumulating intracellular acetyl-CoA *via the* TCA cycle, which is an important metabolic pathway for the bioproduction of IA (Zhou et al., 2012; Ng et al., 2020).

Previous studies on the metabolic engineering of *Y. lipolytica* for the bioproduction of IA were previously performed. Blazeck et al. (2015)



(caption on next page)

Fig. 3. Metabolic pathway of *Yarrowia lipolytica* to produce high-value biochemicals. Abbreviations – HK: hexokinase; GPI: glucose-6-phosphate isomerase; PFK: phosphofructokinase; FBA: fructosebisphosphate aldolase; DAHP: 3-deoxy-D-arabino-heptulosonic acid 7-phosphate; DAHPS: 3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthase; PK: pyruvate kinase; TPI triosephosphate isomerase; G6PDH: glucose-6-phosphate dehydrogenase; 6PGL: 6-phosphogluconolactonase; 6PGDH: 6-phosphogluconate dehydrogenase; 6-PGL: 6-phosphogluconolactone; 6-PL: 6-phosphogluconate; Ru5P: ribulose-5-phosphate; R5P: ribose-5-phosphate DHA: dihydroxyacetone; DHAP: dihydroxyacetone phosphate; TAL: tyrosineammonia lyase; GYC1: glycerol dehydrogenase; DAK1: dihydroxyacetone kinase; DHQ: dihydroquercetin; 4-CC: 4-coumaroyl-CoA; STS: resveratrol synthase; 4-CL: 4-coumaryl ligase; CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavonoid-3-hydroxylase; NOMT: Naringenin-*O*-methyltransferase; UbiC: chorismite lyase; ADH: alcohol dehydrogenase; AS: glycosyltransferase; CAR: carboxylic acid reductase; PT1: prenyltransferase; CNS1: oxidoreductase / dehydrogenase enzyme complexes; CNS2: metal-dependent phosphohydrolases; GLK1: glucokinase; PGM1: phosphoglucomutases; GUT1: glycerol kinase; PEP: phosphoenolpyruvate; CA: citric acid; Iso-CA: isocitric acid; α-KG: α-ketoglutarate; SA: succinic acid; OAA: oxaloacetic acid; CIT1: mitochondrial citrate synthase; ACO2: mitochondrial aconitase; IDH2: mitochondrial isocitrate dehydrogenase; KGDH: ketoglutarate dehydrogenase; LSC1: succinate-CoA ligase; SDH: succinate dehydrogenase; FUM1: fumarase; MDH1: mitochondrial malate dehydrogenase; ACL1: ATP-citrate lyase; MTT: mitochondrial tricarboxylic transporter; CAD: cis-aconitate decarboxylase; HMG-CoA:; DMAPP: dimethylallyl pyrophosphate; IPP: isopentenyl pyrophosphate; NPP: neryl pyrophosphate; GPP: geranyl pyrophosphate; FPP: farnesyl pyrophosphate; GGPP: geranylgeranyl pyrophosphate; GGOH: geranylgeraniol; ERG10: acetoacetyl-CoA thiolase; ERG13: hydroxymethylglutaryl-CoA synthase; HMGR: hydroxymethylglutaryl-CoA reductase; ERG12: mevalonate kinase; ERG8: phosphomevalonate kinase; ERG19: diphosphomevalonate decarboxylase; IDI1: isopentenyl-diphosphate delta-isomerase; NDPS1: neryl diphosphate synthase; LS: limonene synthase; ERG20: farnesyl diphosphate synthase; LIS: linalool synthase; FS: farnesene synthase; ERG9: squalene synthase; ERG1: squalene monooxygenase; MAS: multifunctional amyrin synthase; GGS1: geranylgeranyl pyrophosphate synthase; DPP1: diphosphate phosphatase; LPP1: lipid phosphate phosphatase; CARB: phytoene dehydrogenase; CARRB: phytoene synthase / lycopene cyclase; CrtZ: β-carotene hydroxylase; CCD2: carotenoid-cleaving dioxygenase; ALD: aldehyde dehydrogenase; LIP2: lipases; POT1: peroxisomal thiolase; POX1-6: six different acyl-CoA oxidases; MFE1: multifunctional enzyme; ICL1: isocitrate lyase; MLS: malate synthase; CIT2: peroxisomal citrate synthase; ACO1: peroxisomal aconitase; MDH3: peroxisomal malate dehydrogenase.

successfully demonstrated the capacity of this strain for IA production by heterologous expression of the cis-aconitic acid decarboxylase (CAD) gene from *A. terreus*. This decarboxylase catalyze the conversion of cis-aconitic acid to IA (Blazeck et al., 2015). Up to 4.6 g/L of the product was obtained *via* metabolic engineering, enzyme localization, and bioreactor scale-up. Nevertheless, it was also found that the overexpression of mitochondrial cis-aconitate transporter (MTT) could further boost the IA titer yield up to 22.03 g/L (Zhao et al., 2019).

An IA titer yield of 54.55 g/L was obtained in a 5 L bioreactor by expressing the cis-aconitic acid decarboxylase (CAD) gene from *A. terreus* in either the peroxisome or the cytosol and overexpressing all the 10 necessary genes involved in the production pathway of acetyl-CoA (Rong et al., 2022). Recently, the reprogramming of the metabolic engineering systems within *Y. lipolytica* was reported by Fu et al. (2024). The enhancement in IA titer yield was achieved through modular approach strategies: Deletion of DGA1, DGA2, LRO1, and ARE1 to block the formation of sterol esterification (SE) and TAG; Deletion of isocitrate dehydrogenase (IDP) and isocitrate lyase (ICL1) to block the accumulation of α-KG and glyoxylate, respectively; Overexpression of MTT, MDT, and CAD genes to increase the IA titer yield significantly (Fu et al., 2024). At the end of the day, 130.1 g/L and 94.8 g/L of IA were successfully obtained in a 1 L bioreactor and a 50 L bioreactor on a semi-pilot scale, respectively. This was by far the highest product concentration achieved *via the* metabolic engineering of yeast cells.

5.1.5. *α-Ketoglutarate*

α-Ketoglutarate (α-KG) is a short-chain carboxylate which is widely used for the production of fine chemicals in pharmaceutical, petrochemical, food, and cosmetic industries (Guo et al., 2016). It is an intermediate in the TCA cycle that plays an important role in various cellular metabolic pathways, especially in the regulation of the balance between nitrogen and carbon in most microorganisms (Fendt et al., 2013). The industrial production of α-KG is currently done *via* chemical processes involving the use of oxalic acid, diethyl esters, and succinic acid to give a 75% titer yield (Stottmeister et al., 2005). However, this approach still suffers from low product titer and productivity, as well as high exposure to hazardous chemicals and the formation of environmental hazards during the manufacturing processes (Yin et al., 2015). Thus far, industrial production of biobased α-KG has not been established to date.

Although different kinds of microorganisms were previously used as hosts for the production of biobased α-KG, the growth of *Y. lipolytica* requires the simplest nutritional demand, which results in more efficient production of α-KG (Otto et al., 2011). For instance, overexpressing pyruvate dehydrogenase complex (PDHC) encoded by PDA1 in *Y. lipolytica* WSH-Z06, a natural α-KG producer, successfully improved the production of α-KG up to 43.3 g/L with a yield of 3.7 g/g (Guo et al., 2014). PDA1 is a gene involved in thiamine pyrophosphate (TPP) binding and plays a crucial role in the production of α-KG.

Zhou J. et al. successfully enhanced the α-KG production using the same parental strain of *Y. lipolytica* through alteration of the acetyl-CoA metabolism (Zhou et al., 2012). The ATP-citrate lyase gene (ACL) from *Mus musculus* and acetyl-CoA synthase (ACS1) from *Saccharomyces cerevisiae* were co-expressed together to give the α-KG titer as high as 56.5 g/L in 3 L-bioreactor. Thus far, the highest bioproduction α-KG was demonstrated by Yovkova et al., 2014. 186 g/L of the product was successfully obtained in 117 h through overexpression of both isocitrate dehydrogenase (IDP1) and pyruvate carboxylase (PYC1) genes, which are responsible for the conversion of ICA to α-KG and pyruvate to oxaloacetic acid, respectively.

Apart from the metabolic engineering strategy, bioprocess technology approach has also been explored to further enhance the bioproduction of this molecule. For instance, optimization of medium composition and operating conditions significantly boosted up the α-KG titer up to 86 g/L from biodiesel waste, demonstrating that waste valorization can be effectively integrated into this system (Kamzolova and Morgunov, 2020). The use of mixed renewable carbon sources (rapeseed oil and glycerol) was also investigated by Rymowicz's research group back in 2023, allowing 82.4 g/L of α-KG to be obtained after 6 days fermentation in 5 L bioreactor (Tomaszewska-Hetman et al., 2022). Recently, it was also reported that thiamine concentration was identified as a critical factor for improving α-KG purity by reducing pyruvic acid byproduct formation. An optimized thiamine supplementation approach combined with a continuous feeding strategy was successfully boost up the α-KG production from crude glycerol up to 117 g/L, offering an efficient utilization of crude glycerol waste for the production of high-value biochemicals (Tomaszewska-Hetman et al., 2025).

5.2. *Mevalonate pathway*

Terpenoids are one of the biggest classes of natural products which play a pivotal role in the production of pharmaceuticals, high-value fine chemicals, flavours, fragrances, and high-quality liquid fuel alternatives (Moser and Pichler, 2019). Terpenoids are commonly comprised of isoprene units, which could be furtherly classified into hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, tetraterpenoids, and another form of polyterpenoids. Despite their extensive applications in the aforementioned fields, the bioproduction of terpenoids still suffers from several limitations, such as high production cost, low product titer, and their limited existence in nature. Thus, efficient and sustainable bioproduction of these compounds is highly desirable to meet the criteria for large-scale industrial production. *Yarrowia lipolytica*, fortunately, possesses its endogenous mevalonate (MVA) pathway (Gao et al., 2016, 2017b), which is crucially involved in the biosynthesis of terpenoids (Fig. 3) (Liu et al., 2020b; Ma et al., 2019). Two crucial building blocks to obtain terpenoids *via the* MVA pathway are isopentenyl diphosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP). The two compounds will facilitate as precursors for a bioproduction of terpenoids catalyzed by different terpene synthases (Jongedijk et al., 2015).

All of these results suggested that this cell line has the potential to synthesize liposoluble chemicals in an economically feasible manner (Huang et al., 2018; Plácido and Capareda, 2016). Several approaches have been considered to obtain large-scale production of terpenoids in *Y. lipolytica*, which include but are not limited to optimizing the mevalonate pathway, downregulating the competing pathway, and optimizing the fermentation process (Table 6).

5.2.1. *Squalene*

Squalene is an acyclic triterpene that has been widely used in the food, pharmaceutical, and cosmetic industries (Kim and Karadeniz, 2012; Narayan Bhilwade et al., 2010). The market of this molecule is expected to reach $204 million in 2024, with an annual growth rate of 7.8% (Meng et al., 2020). The availability of squalene in nature is very limited since it is mainly sourced from the liver oil of deep-sea sharks and plant seeds (Huang et al., 2018). Therefore, it is challenging to obtain this compound in high purity due to the various impurities. As a result, downstream processing becomes more inefficient and costly (Chang and Keasling, 2006). The use of *Y. lipolytica* as a host to produce biobased squalene was first reported by Huang et al. (2018). The overexpression of endogenous hydroxy methyl glutaryl-CoA reductase encoding HMGR1, ATP citrate lyase encoding ACL1, and acetyl-CoA synthetase from *Salmonella enteria* could synergistically improve the product titer to 10 mg/g DCW (Huang et al., 2018).

Conversely, NADPH recycling from the mannitol cycle was also proven to enhance the production of squalene significantly (Liu et al., 2020b). These were done through the overexpression of mannitol dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzymes, citrate lyase, and succinate semi-aldehyde dehydrogenase encoding MnDH, GND2, MAE1, IDP2, and UGA2 genes, respectively, to give a total product titer of 0.5 g/L with 32.6 mg/g DCW yield. The overexpression of HMGR1 together with diacylglycerol acyltransferase encoding DGA1 was also reported to successfully enhance the product titer up to 0.732 g/L with 49.61 mg/g DCW yield (Tang et al., 2021).

Recently, the highest production of squalene in *Yarrowia lipolytica* was reported by Xu et al. (2024) without adding any inhibitor, and this was achieved by combining 2 approaches: first is to remodel the homologous recombinant by knocking out the KU70 gene. This was subsequently followed by the integration of the whole mevalonate pathway into the chromosome and overexpression of necessary genes within (ERG10, ERG13, tHMGR1, ERG12, ERG8, ERG9, IDI, and ERG20). Lastly, deletion of MLS1 and ERG5 genes was also performed (Xu et al., 2024). With all these genetic modifications, a production titer of 35 g/L of squalene was successfully achieved in a fed-batch 5 L bioreactor.

On the other hand, the use of terbinafine, an inhibitor to prevent the conversion of squalene to 2,3-oxidosqualene *via* oxidation with squalene monooxygenase (ERG1), was also explored before. Although it is costly, the addition of an ERG1 inhibitor could significantly enhance squalene production. This approach successfully gave a product titer of 51 g/L of in a fed-batch 5 L bioreactor. This is the highest production titer of squalene in *Y. lipolytica* reported thus far.

The highest squalene titer of 3.43 g/L was also recently achieved in a 5 L fed-batch fermenter by consolidating metabolic flux towards the squalene, in tandem with the expression of native ATP-binding cassette transporter (SNQ2) and an engineered oxysterol-binding protein for squalene export (Chai et al., 2025).

5.2.2. *Limonene*

Limonene is a monocyclic monoterpene that has been widely used for flavours and fragrances due to its well-known citrus-like odour properties (Jongedijk et al., 2015; Oswald et al., 2007; Serra et al., 2005). Their application in pharmaceutical industries is also important since they serve as a precursor for the production of carvone, menthol, and perillyl alcohol (POH) (Alonso-Gutierrez et al., 2013; Oswald et al., 2007). Limonene usually exists in natural plants in the form of 3 different isomers: dL-limonene, l-limonene, and D-limonene, which is impossible to obtain *via* chemical synthesis.

D-limonene is specifically classified as a GRAS compound by the US Food and Drug Administration and has been utilized as a fragrance ingredient and flavouring additive in the food and cosmetic / perfumery industries for the past decades (Mamidipally and Liu, 2004). Naturally produced limonene is mainly obtained from the byproducts of orange juice production or *via* the fractionation of essential oil (Du et al., 2014; Oswald et al., 2007). Considering the manufacturing process, which still requires harsh reaction conditions and is inefficient, an alternative method to produce limonene is highly desired.

Limonene could be produced through the metabolic engineering of microorganisms *via* the MVA pathway, which clearly serves as a better platform to overcome these issues. *Yarrowia lipolytica*, fortunately, can be selected as a suitable host to facilitate the production of this compound. Geranyl diphosphate (GPP) acts as a direct crucial precursor for the biosynthesis of limonene. It could be endogenously produced from IPP and DMAPP, catalyzed by farnesyl / geranyl diphosphate synthase (ERG20). Further conversion of GPP by limonene synthase (LS) will result in the formation of limonene.

Cao et al. (2016) employed metabolic engineering to enable *Y. lipolytica* to heterologously produce limonene. The group constructed a limonene synthetic pathway harbouring neryl diphosphate synthase (NDPS1) and limonene synthase (LS) from *Agastache rugosa* and *Solanum lycopersicum*, respectively. NDPS1 is an exogenous gene that was introduced to convert IPP and DMAPP to neryl pyrophosphate (NPP). NPP will be further converted to limonene by LS. Additionally, HMGR1 and mevalonate kinase (ERG12) genes involved in MVA pathways were also overexpressed. This strategy resulted in a titer of up to 23.56 mg/L limonene after 3-4 days of cultivation in the shake flask (Cao et al., 2016).

This number, however, is still considered low for industrialization. To solve this issue, another copy of the LS gene was introduced, and this newly engineered strain was used in the 6-day fed-batch fermentation process. The new approach successfully enhances the production concentration up to 165 mg/L (Cheng et al., 2019).

Different substrates as carbon sources were also explored to reduce the cost of limonene production further. Xylose and lignocellulosic hydrolysate were utilized to enhance the production of limonene, giving up to 20.57 mg/L of product titer. This was achieved by introducing heterologous genes xylitol dehydrogenase (XDH) and xylose reductase (XR), together with the native xylose kinase (XK) gene, which were all derived from *S. stipites*. tNDPS1 and tLS encoding orthogonal limonene biosynthetic pathway was also overexpressed synergistically (Yao et al., 2020).

In a contribution to minimize and develop novel solutions for managing food waste, a combined approach of metabolic engineering and the use of waste cooking oil as a substrate was also previously explored to give the *d*- and *L*-limonene product titers of 2.514 and 2.723 mg/L, representing the first report on the sustainable development process for the bioproduction of these molecules from waste (Pang et al., 2019). Subsequently, the same group finally came out with a new metabolic engineering strategy, which is to further engineer the metabolic pathway and optimized the medium composition. In addition to the overexpression of homologous HMGR1, heterologous *L*-limonene synthase gene from *Mentha spicata* and *D*-limonene synthase gene from *Citrus limon* (Pang et al., 2019), they started to further overexpress the IDI1 and tNDPS1 into the gene cascade inside the plasmid. As a result,

Table 6
Bioproducts derived from the mevalonate pathway.

Product	Substrate	Engineering Strategy	Titer (mg/L)	Yield (mg/g Substrate)	Scale	Time (h)	Ref.
Squalene	Glucose	HMG1↑, SeACS*↑,ACL1↑, ΔMLS, ΔCIT2	NA	NA	Flask (50 mL)	120	(Huang et al., 2018)
Squalene	Glucose	MnDH2↑, ACL2↑, HMG1↑, SQS↑	500	12.57	Flask (30 mL)	188	(Liu et al., 2020)
Squalene	Glucose	HMG1↑, DGA1↑, Additional use of terbinafine	731	36.55	Flask (50 mL)	72	(Tang et al., 2021)
Squalene	Glucose	ERG10↑, ERG13↑, tHMG1↑, ERG12↑, ERG8↑, ERG9↑, IDI↑, ERG20↑, ΔERG5, ΔMLS1	35,000	NA	Fed-Batch Bioreactor (2.4 L)	144	(Xu et al., 2024)
Squalene	Glucose	tHMG1↑, ERG12↑, ERG20↑, ERG9↑, IDI↑, POT1↑, POX1↑, ACL1↑, EcAAD↑, +terbinafine	51,000	NA	Fed-Batch Bioreactor (2.4 L)	156	(Ning et al., 2024)
Squalene	Glucose	YHM2↑, AMPD↑, ACL↑, AtoB↑, HMGS↑, HMGR↑, EfMvaS↑, ERG12↑, ERG8↑, ERG9↑, IDI↑, ERG20↑, SNQ2↑, OSH3↑	3430	NA	Fed-Batch Bioreactor (1.8 L)	240	(Chai et al., 2025)
Limonene	Glucose	SlNDPS1↑, ArLS↑, HMG1/HMG2↑, ERG12↑	23.56	1.178	Flask (50 mL)	72	(Cao et al., 2016)
Limonene	Glucose or Glycerol	Ar(*t*)LS↑, SltNDPS1↑, HMG1↑, ERG12↑	165.3	NA	Fed-Batch Bioreactor (0.85 L)	144	(Cheng et al., 2019)
Limonene	WCO	Cl(*d*)LS↑,Ms.(*l*)LS↑, HMGR1↑	2.514–2.723	NA	Fed-Batch Bioreactor (2 L)	216	(Pang et al., 2019)
Limonene	Xylose	SsXR↑, SsXDH↑,SsXK↑, HMG1↑, ERG12↑, SltNDPS1↑, Ar(*t*)LS↑	20.57	1.03	Flask (50 mL)	72	(Yao et al., 2020)
Limonene	WCO	Cl(*d*)LS↑,Ms.(*l*)LS↑, HMGR1↑, IDI↑, tNDPS↑	83.06–91.24	NA	Flask (50 mL)	120	(Li et al., 2022)
Linalool	Glucose	AaLIS↑, ERG20↑, HMG1↑, IDI↑, ERG12↑	6.96	0.348	Flask (50 mL)	48	(Cao et al., 2017)
Linalool	Sucrose	AaLIS↑HMG1↑, ERG12↑, ERG20↑, CrGPPS↑, ΔDGK1	109.6	5.48	Flask (50 mL)	168	(Taratynova et al., 2023)
α-Farnesene	Glucose & Fructose	tHMG1↑, IDI↑, ERG20↑, OptFS↑	259.98	NA	Fed-Batch Bioreactor (1 L)	120	(Yang et al., 2016)
α-Farnesene	Glucose or Glycerol	OptFSLERG20↑, Sc-tHMG1↑, IDI↑, HMGR↑, ERG19↑, ΔPEX10	2570	NA	Fed-Batch Bioreactor (2 L)	120	(Liu et al., 2020a)
α-Farnesene	Glucose	MdFS- ERG20↑, ERG12↑, IDI↑, ERG8↑, ERG19↑, GPPS↑,EcAtoB↑, BpHMGR↑	25,550	NA	Fed-Batch Bioreactor (0.8 L)	120	(Liu et al., 2019)
α-Farnesene	Oleic Acid	MdFS-ERG20↑, ERG12↑, VHb↑	10,200	100	Fed-Batch Bioreactor (4 L)	168	(Liu et al., 2021b)
β-Farnesene	Oleic Acid	AaFS↑, AtoB↑, HMGR1↑, HMGS↑	35,200	170	Fed-Batch Bioreactor (4 L)	216	(Liu et al., 2022b)
β-Farnesene	Corn Stover Lignocellulosic Hydrolysate	AaFS↑, HMGR1↑, ERG10↑, ERG12↑, ERG13↑, ERG8↑, ERG19↑, ERG20↑	7380	75	Fed-Batch Bioreactor (2 L)	144	(Bi et al., 2022)
Amyrin	WCO	HMG1↑, ERG9↑, ERG1↑, ERG20↑, CrMAS↑	100 (α-amyrin); 20 (β-amyrin)	NA	Flask (50 mL)	120	(Kong et al., 2022)
Lycopene	Glucose	PacrtB↑, PacrtI↑, GGS1↑, HMG1↑, ΔPOX1-6, ΔGUT2	NA	NA	Fed-Batch Bioreactor (0.6 L)	192	(Matthäus et al., 2014)
Lycopene	Glucose	PacrtE↑, PacrtB↑, PacrtI↑	6.2	0.31	Flask (50 mL)	192	(Zhao et al., 2017)
Lycopene	Glucose	PacrtE↑, PacrtB↑, PacrtI↑, AMPD↑, GPD1↑, DGA1↑, DGA2↑, HMG1↑, IDI↑, ACL1/ACL2↑	745	NA	Fed-Batch Bioreactor (2 L)	120	(Zhang et al., 2019b)
ß-Carotene	Glucose	McCarB↑, McCarRP↑, GGS1↑, tHMG1↑, ERG10↑, ERG13↑, ERG19↑, ERG12↑, ERG8↑, ERG20↑, IDI↑	4000	17	Fed-Batch Bioreactor (1.35 L)	264	(Gao et al., 2017b)
ß-Carotene	Glucose	McCarB↑, McCarRP↑, GGS1↑	6500	36	Fed-Batch Bioreactor (2 L)	122	(Larroude et al., 2018)
ß-Carotene	Glucose	McCarB↑, McCarRP↑, GGS1↑, HMG1↑, ERG13↑	4500	20	Fed-Batch Bioreactor (2 L)	156	(Zhang et al., 2020)
ß-Carotene	Glucose	BtCarB↑, BtCarRP↑, GGS1↑, tHMG1↑	1700	NA (170 mg, based on initial glucose)	Fed-Batch Bioreactor (5 L)	120	(Liu et al., 2021a)
ß-Carotene	Glucose	XdcrtI↑, XdcrtE↑, XdcrtYB↑, CpACC1↑, tHMG1↑	2700	NA (90 mg, based on initial glucose)	Fed-Batch Bioreactor (2 L)	168	(Jing et al., 2023)
ß-Carotene	Glucose	GGS1↑, HygR↑, McCarB↑, McCarRP↑, Δhxt	4101	0.11	Flask (10 mL)	240	(Rafieenia et al., 2025)
Crocetine	Glucose	CrtE↑, CrtB↑, CrtI↑, CrtZ↑, CCD2↑, ALD↑	30.17	NA	Flask (10 mL)	168	(Zhou et al., 2025)
Geranylgeraniol	Glucose	ScLPP↑, ScDPP↑, PaGPPS↑, tHMG1↑, ERG20↑, LmPK↑, EcALDH↑, EcAAD↑, ScCAT2↑, CkPTA↑, ERG9↓	3350	41.83	Flask (50 mL)	120	(Wang et al., 2024a)

83.06 and 91.24 mg/L of *l*- and *D*-limonene was successfully obtained after 5 days fed-batch fermentation (Li et al., 2022).

5.2.3. Linalool

Linalool, an acyclic monoterpene alcohol, is a significant primary component in various essential oils. It constitutes nearly 70% of the terpenoids responsible for floral scents and has been extensively used in the fragrance, flavour, and food industries (Cao et al., 2017). More importantly, it also serves as a crucial precursor for the synthesis of citronellol, farnesol, vitamin A, vitamin E, and ionones. Apart from its aromatic contributions, linalool also possesses noteworthy properties, exhibiting antifungal, antimicrobial, and insecticidal characteristics (Aprotosoaie et al., 2014; Beier et al., 2014; Herman et al., 2016).

Similarly, the bioproduction of linalool in microorganisms could also be done *via* the MVA pathway. The use of *Y. lipolytica* as a host to produce this compound was previously done by Cao et al., 2017. Since this cell line could not endogenously produce linalool, a codon-optimized linalool synthase gene from *Actinidia arguta* (LIS) was introduced into the host, together with the overexpression of the ERG20 gene. This approach was proven to enhance the linalool product titer up to 6.96 mg/L (Cao et al., 2017).

The highest product titer of linalool thus far was reported recently in 2023 by Evgeniya Yuzbasheva's group (Taratynova et al., 2023). The same LIS from *A. arguta* was introduced synergistically with the overexpression of HMGR1, ERG12, and ERG20 genes. Additionally, the deletion of diacylglycerol kinase (DGK1) and the introduction of GPP synthase from *Catharanthus roseus* (CrGPPS) fused with LIS could further boost linalool production. 0.11 g/L of the product was successfully obtained after 7 days of fermentation, becoming the highest limonene production in *Y. lipolytica* that has been reported so far.

5.2.4. Farnesene

α-Farnesene is one of the acyclic sesquiterpenes which belong to a large group of isoprenoid family. This molecule is generally found naturally in apple peels and has a pivotal role in plant defence mechanisms (Nieuwenhuizen et al., 2010; Yang et al., 2011). Its isomers also act as chemical signalling particles, which are useful in various functions in plants, such as predation response, pollination, and seed dispersal (Köllner et al., 2009). α-Farnesene has been widely used in the cosmetic and pharmaceutical industries and has also been established as a building block for the manufacturing of biofuel due to its high energy density and low hygroscopicity properties (Yang et al., 2016).

Despite these achievements, the availability of this compound is still very limited due to its dependence on plant growth. Furthermore, the commonly used plant extraction manufacturing process for α-farnesene production is also often considered unsustainable (Asadollahi et al., 2010; Asadollahi et al., 2009; Mirzaee et al., 2016). Alternative approaches have been developed to further escalate the production of α-farnesene *via* engineering of the metabolic pathway. Similarly, this could also be initiated through the conversion of IPP and DMAPP to GPP *via* the MVP pathway, followed by a further reaction to convert GPP to arsenal pyrophosphate (FPP), which will be the precursor for the α-farnesene production.

The use of *Y. lipolytica* as a host for the α-farnesene production was first explored by Yang et al. (2016) to give the maximum product concentration up to 259.98 mg/L with a yield of 33.98 mg/g DCW in a 1.5 L fed-batch bioreactor (Yang et al., 2016). This titer was successfully achieved through heterologous expression of truncated HMGR1 gene from *Saccharomyces cerevisiae*, together with endogenous overexpression of ERG20, IPP isomerase (IDI), and codon-optimized α-farnesene synthase (OptFS). Further overexpression of endogenous HMGR1 and acetoacetyl-CoA thiolase (ERG10) genes could further enhance the α-farnesene product titer by 3.5 times, giving the maximum concentration of 2.57 g/L (34 mg/g DCW) *via* fed-batch fermentation using both glycerol and glucose as the carbon sources (Liu et al., 2020a).

The highest production of α-farnesene was reported by Jin Hou's group in 2019, in which 25.55 g/L of this molecule was successfully obtained after 12 days of fed-batch fermentation (Liu et al., 2019). A novel approach was explored, which is to overexpress the MVA pathway and codon-optimized α-farnesene synthase gene from apple seed by non-homologous end-joining (NHEJ) mediated integration into the host. Particularly, acetyl-CoA acetyltransferase from *Escherichia coli* and NADH-dependent HMGR1 from *Bordetella petrii* were introduced together with the overexpression of endogenous ERG12, ERG8, ERG 19, IDI, GPPS, and ERG20. This product titer has also been reported as the highest terpenoid titer reported in *Y. lipolytica*.

Since *Y. lipolytica* is well-known for its ability to grow in lipid feedstocks (*i.e.* animal fats and plant oils), a new approach to produce α-farnesene using lipid as the substrate was also explored. By overexpression of *Vitreoscilla* hemoglobin (VHb) membrane protein, FSERG20, and ERG12 genes, 10 g/L of product was obtained, yielding 0.1 g of α-farnesene / g oleic acid, in fed-batch fermentation (Liu et al., 2021b). This report opens up new perspectives for the bioproduction of terpenoids using alternative substrate/carbon sources.

β-farnesene, an isomer of α-farnesene, is a valuable compound that has been widely used as pesticides precursors, surfactants, lubricants, and cosmetics. Recently, it has also been reported to serve as a precursor for the synthesis of Vitamin E. However, its natural availability remains very limited, while chemically produced β-farnesene often suffers from high impurity levels and high production cost, justifying the needs for microbial production of this molecule. Biosynthesis of β-farnesene in *Y. lipolytica* was previously reported in 2022 by Jin Hou's group. Screening of farnesene synthase was conducted, and later it was revealed that β-farnesene synthase from *Artemisia annua* (AaFS) enabled the conversion of oleic acid to this molecule. Combined with the overexpression of other genes involved in the mevalonate pathway, up to 35.2 g/L of product was successfully obtained at the end of 9 days fed-batch fermentation (Liu et al., 2022b).

In the same year, the use of AaFS was also reported to convert corn stover lignocellulosic hydrolysate to β-farnesene. Similar strategy was implied, involving the overexpression of genes within the mevalonate pathway as well as increasing the copy number of HMG-CoA reductase and AaFS (Bi et al., 2023). As a result, 7.38 g/L of product was formed after 6-days of fed-batch fermentation in 2 L bioreactor. The same research group later explored additional approaches to further enhance the β-farnesene titer by optimizing the medium composition and adjusting bioreactor parameters (e.g feeding time point, initial substrate and cell density concentration, and nitrogen-limited condition). Under optimal conditions, 22 g/L β-farnesene was produced in 8 days (Bao et al., 2024). Overall, these results suggest that oil-based media are preferable for future β-farnesene production, and that prolonged fermentation could further increase the product titer as well.

5.2.5. Amyrin

Amyrin is a type of triterpenoid widely used for the production of pharmaceuticals, high-value fine chemicals, and bioactive compounds (Askari et al., 2018; Neto et al., 2021; Seki et al., 2008). Chemically produced amyrins cannot give the target product high enantioselectivity, and their manufacturing process still suffers from high energy consumption and low efficiency. Biobased production of amyrins *via* the engineering of metabolic pathways is, therefore, highly preferred to solve these problems.

The use of *Y. lipolytica* as a host for amyrins production was recently reported in 2022 by Jing, K. et al. The native genes responsible for MVA pathway were overexpressed: HMGR1, ERG1, ERG20, and ERG9 encoding squalene synthase. More importantly, heterologous gene amyrin synthase (CrMAS) from *Catharanthus roseus* was also introduced. After optimizing the fermentation operating conditions, 100 and 20 mg/L of α- and β-amyrins were successfully obtained, respectively (Kong et al., 2022). This was by far the only research article on the bioproduction of amyrin in *Y. lipolytica* which has ever been reported.

5.2.6. Geranylgeraniol

Geranylgeraniol (GGOH) is an acyclic diterpenoid alcohol naturally found in *Bixa Orellana*, a plant best known as the source of industrial food colouring (De Oliveira Júnior et al., 2019). While serving its purpose as a major component in fragrances, food, and cosmetic industries, this compound also exhibits antibacterial and anti-inflammatory properties, thus effectively promising as an alternative to cure thrombosis, ulcers, and neurasthenia.

Chemically synthesized GGOH still suffers from low enantioselectivity and a challenging product separation process, and therefore, biologically-derived GGOH is much preferable. Recently, it has been demonstrated that the bioproduction of GGOH in *Yarrowia lipolytica* successfully gave 3.35 g/L of product, making it the highest reported GGOH titer in a shaking flask to date. This success was mainly achieved through overexpressing heterologous phosphatase genes from *Saccharomyces cerevisiae:* ScDpp1 and ScLpp1 encoding diacylglycerol diphosphate phosphatase and lipid phosphate phosphatase, respectively, as well as downregulating the ERG9 gene responsible for the squalene production and increase the FPP and GPP accumulation *via* metabolic engineering within mevalonate pathway (Wang et al., 2024a).

5.2.7. Lycopene

Lycopene is a value-added bioactive molecule that represents a vast class of carotenoids. Due to its excellent functionality and anti-oxidative, anti-cancer, and anti-inflammatory properties, it has been commercially used in food additives and medicine applications (Amorim-Carrilho et al., 2014; Ciccone et al., 2013; Khuda-Bukhsh et al., 2014). This molecule is hydrophobic, and it is naturally present in plants like tomatoes, papaya, watermelon, red oranges, and apricots, but it is limited in availability (Nambou et al., 2015). Moreover, the current extraction and manufacturing process still encounters several challenges, which can be attributed to their unsustainability, low titer yield, costly production process, and inefficient processing time.

To further solve these issues, lycopene production *via* fermentation has gained a lot of attention for the past 10 years. Within microorganisms, lycopene is mainly located in lipid bodies due to its hydrophobic properties. Therefore, *Y. lipolytica* is considered a strong host candidate compared with the other lycopene-produced cell lines because its lipid bodies could store a huge number of this kind of hydrophobic molecules. This cell line has also emerged as a promising host for lycopene and other carotenoid production due to its ability to produce a high level of acetyl-CoA, a precursor for both lipid and carotenoid production *via* Fatty Acid biosynthesis pathway and MVA pathway, respectively.

A range of genetic engineering approaches has been conducted further to improve the production of lycopene in *Y. lipolytica*. In 2014, for instance, Mathhaus F. et al. successfully engineered this yeast strain to give a yield of 16 mg/g DCW in the fed-batch fermentation process. This achievement was accomplished by introducing crtB and crtI codon-optimized genes from *Pantoea ananatis*, deleting the endogenous six acyl-CoA oxidases (POX1-6) and glycerol kinase (GUT2) genes, as well as overexpressing geranylgeranyl pyrophosphate synthase (GGS1) and HMGR1 within the host (Matthäus et al., 2014). Hua's group also reported the introduction of heterologous crt genes in 2017. When all *crtB, crtE,* and *crtI* were all expressed, 6.2 mg/L of lycopene was successfully obtained (1.7 mg/g DCW yield) after 8 days of cultivation in the shaking flask. This group also investigated the effect of the insertion of different copies of crt genes from *P. agglomerans* and overexpression of the AMP deaminase-encoding gene (AMPD) from *Y. lipolytica*. Optimization using a 5 L fed-batch bioreactor successfully gave 745 mg/L of product with 16.1 mg/g DCW yield (Zhang et al., 2019b). This report justifies that by modifying the copy numbers of the crt genes, plasmid design could significantly enhance the FPP effectiveness for the bioproduction of lycopene.

5.2.8. β-Carotene

β-Carotene is a naturally occurring pigment that belongs to the class of carotenoids, which are organic compounds mainly discovered in algae, plants, and certain microorganisms. It is also widely known as a precursor of vitamin A and could serve as a strong anti-oxidant which protects cells from damage caused by harmful inhibitors and environments (Gao et al., 2017b; Liu et al., 2021a). This compound is commonly used as a natural food colouring agent and supplement because of its potential health benefits, including supporting immune function.

Due to its potential and health benefits, the β-carotene market has been projected to significantly increase from USD 432.2 million in 2015 to USD 600 million by 2024 (Larroude et al., 2018). The conventional method for β-carotene production is usually performed through *de-novo* chemical synthesis *via* food and vegetable extraction. This approach, however, suffers from low product yield, low productivity, and high operating costs; thus, it will not be suitable for larger-scale applications (Bogacz-Radomska and Harasym, 2018).

With the growing demand for naturally-product high-value chemicals, microbial cell factories have shown up as a promising platform to obtain the biobased β-carotene. Fortunately, the use of *Y. lipolytica* has emerged as one of the great alternatives for the bioproduction of β-carotene in attribution to its metabolic flexibility, robustness, and deference towards genetic manipulation. This species is also well-known for its ability to produce a high amount of acetyl Co-A, a crucial precursor to making this compound *via the* mevalonate pathway.

Many approaches have been carried out to obtain this compound in *Y. lipolytica*. Firstly, Gao et al. (2017a, 2017b) successfully constructed a strain through a series of 12 steps, where 11 of the genes were modified and overexpressed. This includes the corporation of exogenous β-carotene synthesis genes from *Mucor circinelloides*: CarB and CarRP encoding phytoene dehydrogenase and phytoene synthase/lycopene cyclase, respectively. Their investigation revealed that incorporating multiple copies of certain designated genes was crucial to enhance the β-carotene production, giving the product titer as high as 4 g/L *via* fed-batch fermentation (Gao et al., 2017a).

The second method was to optimize the lipid biosynthesis pathway to increase the amount of acetyl-CoA by overexpressing the DGA2 and GPD1 genes and removing the tgl4 and POX1-6 genes, which will subsequently elevate the β-carotene production. Multiple copies of car cassettes harbouring endogenous GSS1 with exogenous CarB and CarRP from *M. circinelloides* were also introduced. As a result, total production of 6.5 g/L β-carotene (90 mg/g DCW and 36 mg/g glucose) was finally achieved after 122 h of cultivation *via* fed-batch fermentation (Larroude et al., 2018).

CRISPR/Cas9 strategy was also previously implied by Qiang Hua's group back in 2020 to obtain 4.5 g/L of β-carotene *via a* fed-batch fermentation process. This success was achieved by introducing 2 copies of CarB, 3 copies of CarRP, and a single copy of each HMGR1, GGS1, and ERG13 genes (Zhang et al., 2020).

Apart from *M. circinelloides*, the use of heterologous β-carotene synthesis genes from other species was also reported. The use of codon-optimized CarB and CarRP from industrial strain *Blakeslea trispora* cooperated with endogenous tHMGR1 and GGS1 genes were previously investigated back in 2021 (Liu et al., 2021a, 2021b). Although the product concentration is not too high, this approach still successfully gave 1.7 g/L of β-carotene (21.6 mg/g DCW yield). Moreover, β-carotene synthesis cassettes harbouring *crt*I, *crt*B, and *crt*YB genes from *Xanthophyllomyces dendrorhous* encoding phytoene desaturase, geranylgeranyl diphosphate synthase, and phytoene synthase/lycopene cyclase, respectively, were also introduced into the genome of *Y. lipolytica.* Concurrent with the corporation of exogenous acetyl-CoA carboxylase (ACC1) from *Cryptococcus podolicus* and endogenous tHMGR1 genes, 2.7 g/L of β-carotene was successfully obtained after 168 h of fed-batch fermentation (Jing et al., 2023).

In terms of optimization of downstream processing, the use of micro-fermentation was investigated in 2020 by Martinez, J.L.'s group, using the same strain that was previously constructed by Larroude et al. in 2017 (Larroude et al., 2018). Through medium optimization, a 50%

enhancement in β-carotene titer yield was observed, giving up to 0.4 g/L of product (Jacobsen et al., 2020). Although the yield is not that high, this invention opened up a new alternative for the production of carotenoids in the micro-fermentation system.

Additionally, a co-culture system was also developed in 2024 by Rodrigo Ledesma-Amaro's group to enhance the β-carotene production by using two different *Y. lipolytica* strains: one is a wild type strain (*Y. lipolytica* H222) which catalyze the conversion of glucose to citric acid (CA); and an ‘upcycle’ strain (*Y. lipolytica* RLA 1072) with a knocked-out HXT gene encoding hexokinase harbouring β-carotene gene cassette (Larroude et al., 2018), which further converts CA to β-carotene. ALE approach was conducted so that this particular strain could only consume CA as the sole carbon source. As their best results, 4101 mg/L of product was obtained at the end of the 10-day co-culture fermentation system. This amount is nearly twice that of the fermentation performed without the co-culture approach (2009 mg/L).

In 2025, the same group also further incorporated CrtZ, CCD2, and ALD genes encoding β-carotene hydroxylase, carotenoid-cleaving dioxygenase, and aldehyde dehydrogenase, respectively, to create another pathway to produce crocetine, a multifunctional apocarotenoid natural product derived from saffron. This group successfully produced 30.17 mg/L of product through this crocetine biosynthesis pathway *via* two-step temperature shift fermentation to significantly enhance the cell growth and enzymatic activity (Zhou et al., 2025).

5.3. Pentose phosphate cycle

The pentose phosphate pathway (PPP) is integral to cellular function and carbon metabolism, performing three critical functions: (i) generating NADPH for cellular redox reactions and biosynthetic processes, (ii) producing ribose-5-phosphate (R5P) for nucleotide biosynthesis, and (iii) providing erythrose-4-phosphate (E4P) as a precursor for the biosynthesis of aromatic amino acids *via* the shikimate pathway (Masi et al., 2021). The PPP is intricately linked to other metabolic pathways, such as glycolysis, TCA cycle, and fatty acid biosynthesis, making it a strategic target for metabolic engineering (Fig. 3). Manipulation of the PPP in *Y. lipolytica* can redirect carbon flux towards the downstream biochemical pathways for secondary metabolites such as flavonoids and

Table 7
Bioproducts derived from the pentose phosphate pathway.

Product	Substrate	Engineering Strategy	Titer (g/L)	Yield (g/g substrate)	Scale	Time (h)	References
Erythritol	Glucose	ΔRAS2, ΔEYD1	220.2	0.6	Bioreactor (2 L)	96	(Liu et al., 2024)
Erythritol	Glucose	ZWF1↑, GND1↑, ER10↑, ER2↑5, ER27↑	190	0.63	Flask (500 mL)	80	(Cheng et al., 2018)
Erythritol	Glucose	ΔMHY1, ΔCLA4, YTH1↑, YTH3↑	171.67	0.56	Fed-batch bioreactor (1.5 L)	72	(Xu et al., 2023)
Erythritol	Glucose	ΔEYD1, ΔCLA4, ΔMHY1, ZWF1, GND1↑, RPE1↑, RPI1↑, TKL1↑, TAL1↑, ER10↑, ER27↑, FBP1↑, TPI1↑, CmALR↑	256	0.58	Fed-batch bioreactor (2 L)	144	(Yang et al., 2024a)
Erythritol	Glucose	↑ZWF1, ↑GND1, ScRSP5, ΔEYD1, ΔMDH2	196	0.65	Bioreactor (22,500 L)	78	(Wang et al., 2020a)
2-PE	Glucose	ΔTYR1, ΔTRP2, ΔTRP3, ΔARO8, ΔARO9, ΔPK, ARO1↑, ARO2↑, ARO3↑, ARO4↑, ARO5↑, ScARO4^{K229L}↑, EcAro^{GS180F}↑, TKT↑, BbXfpK↑, AcXpk↑, PAR4↑, ARO10↑, PHA2↑, ARO7↑, ScARO7^{G141S}::Leu↑	2.43	0.444	Flask (30 mL)	120	(Gu et al., 2020)
2-PE	Glucose	EcAROG^{fbr}↑, EcPheA^{fbr}↑, ARO10↑, PAR4↑, ΔTYR1, TKT↑, ARO1↑, PHA2↑	2.40	0.06	Flask (50 mL)	84	(Qian et al., 2024)
p-Coumaric acid	Glucose Cellulose Hemicellulose	ARO4^{K221L}↑, ScARO3^{K222L}↑, ΔPHA2,XT↑, RgTAL↑	1.04 (glucose) 0.08 (cellulose) 0.07 (hemicellulose)	0.02	Flask (50 mL)	120	(Zhu et al., 2024)
Drupanin	Glucose	ARO1↑, ARO2↑, ARO3↑, ARO4↑, ARO7↑, TAL↑, AtoB↑, EfMvaE↑, EfMvaS↑, MmMK↑, HMGR↑, HMGS↑, ERG12↑, ERG8↑, ERG19↑, IDI↑, AcPT1↑	0.0526	NA	Fed-batch bioreactor (3 L)	240	(Liu et al., 2025)
Cordycepin	Molasses	CNS1↑, CNS2↑, GLK1↑, PGM1↑	2.29	0.053	Flask (50 mL)	120	(Duan et al., 2023a)
Gastrodin	Glucose	DHS1↑, ↑ARO4^{K221L}↑, EcUbiC↑, EcAroG^{G146N}↑, NoCAR↑, BsSFP↑, RhAS↑, ΔPDC1, ΔARO10, ΔPAR4, ΔPHA2, ΔTRP1	13.22	0.33	Fed-batch bioreactor (2 L)	132	(Wu et al., 2024)
Resveratrol	Glucose	FjTAL↑, Pc4CL1↑, VvSTS↑, ΔDGA1	22.5	0.066	Fed-batch bioreactor (2.5 L)	144	(Liu et al., 2022a)
Violacein	Glucose	ΔTYR1, ΔTRP2, ΔTRP3, ΔARO8, ΔARO9, ΔPK, ARO1↑, ARO2↑, ARO3↑, ARO4↑, ARO5↑, ScARO4^{K229L}↑, EcAroG^{S180F}↑, TKT↑, BbXFPK↑, AcXPK↑, TRP5↑, TRP4↑, TRP3↑, TRP2↑, ↑RP1↑, VioA↑, VioB↑, VioC↑, VioD↑, VioE::Leu↑	0.37	0.08	Flask (30 mL)	120	(Gu et al., 2020)
Scutellarin	Glucose	PGM2↑, UGP1↑, ZWF1↑, GND1↑, EcAroG*↑, EcTyrA*↑, RgTAL↑, EbUDPGDH↑, EbF7GAT↑, At4CL3↑, AtCHS3↑, AtCHI1↑, PcFNSI↑, AtATR2↑, VHb↑, SbF6H↑, ΔDGA2	0.7	0.018	Flask (50 mL)	84	(Zhang et al., 2023)
Dihydroquercetin	Glucose	IDH2↑, IDP2↑, VHb↑, SmCPR↑, TAL↑, PAL↑, C4H↑, 4CL↑, CHS↑, CHI↑, GhF3’H↑, SlF3H↑	4200	NA	Fed-Batch Bioreactor (5 L)	144	(He et al., 2025)
Sakuranetin	Glucose	RtTAL↑, Pc4CL↑, PhCHS↑, MsCHI↑, OsNOMT↑, SAH1↑, MET6↑, SAM1↑, ACL1↑, ACC1↑, ExPOXb↑, BsYdp↑	0.344	0.003	Fed-Batch Bioreactor (5 L)	120	(Ge et al., 2024)
Sakuranetin	Glucose	OSNOMT↑, MAT↑, EcMetK↑, MetE↑, SAH1↑, ARO1↑, tyR1↑	0.687	0.009	Flask (50 mL)	168	(Xu et al., 2025)

anthocyanins, thereby enhancing the production of target compounds (Table 7).

5.3.1. *Erythritol*

Metabolic engineering efforts targeting the PPP in *Y. lipolytica* have primarily focused on enhancing the production of erythritol, *i.e.*, (2*R*,3*S*)-butane-1,2,3,4-tetraol, a four-carbon sugar alcohol valued for its low-calorie sweetening properties (Cheng et al., 2018). Due to its minimal impact on glucose and insulin levels upon consumption, erythritol is increasingly being adopted as an alternative sweetener in the pharmaceutical and agro-food sectors. Its production value is expected to be above 150 million USD (Liu et al., 2024). Erythritol is conventionally produced from glucose by osmophilic yeasts (*e.g.*, *Moniliella megachiliensis* and *Candida magnoliae*) (Liu et al., 2024; Yang et al., 2024a). However, recent years have seen the emergence of *Y. lipolytica* as an efficient host for erythritol production, with notable production titers achieved through the use of metabolically engineered strains.

One study focused on enhancing erythritol production involved engineering a high-yielding strain (CA20) to form a stable yeast cell line with reduced erythritol degradation through deletion of the RAS2 and EYD1 genes encoding GTP binding protein and erythritol dehydrogenase, respectively. This resulted in an erythritol yield of 161.5 g/L in a 125 mL shake flask after 132 h using YPD media with 200 g/L glucose. When complemented with a carbonized walnut shell and carbonized sugarcane bagasse biochar, the glucose metabolism of the engineered strain was significantly enhanced, resulting in higher erythritol yield (220 g/L) within 72 h in a 2 L fermenter (Liu et al., 2024). In another approach, overexpression of genes encoding for erythrose reductases (ER) and two PPP enzymes, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, resulted in an erythritol yield of 190 g/L within 80 h (Cheng et al., 2018).

Morphological and glucose transporter engineering strategies have also proven effective in boosting erythritol production in *Y. lipolytica*. MHY1 gene deletion from a high-yielding strain (CGMCC7326) led to a stable yeast cell line, which was hypha-free. This modification was complemented with hexose transporter YTH1 and YTH3 overexpression for enhanced glucose uptake. Consequently, 171.7 g/L of erythritol was achieved in 3 days in a 1.5 L fermenter (Xu et al., 2023). A more recent study employed a CRISPR-Cas9 system for targeted and marker-free genetic manipulation of *Y. lipolytica* to enhance its erythritol productivity. The researchers first altered carbon flux by inhibiting the erythritol degradation pathway. Subsequently, through target screening and promoter engineering, they identified eleven crucial gene targets and a robust promoter, 8UAS1B$_{\text{XPR2}}$-P$_{\text{TEFin}}$, to direct metabolic flux towards the target product molecule. Finally, employing modular pathway engineering and morphological optimization, they successfully increased erythritol production to 256 g/L, which is the highest titer ever reported in *Y. lipolytica* (Yang et al., 2024a).

In a separate study, Wang et al. implemented metabolic engineering strategies to alleviate challenges pertaining to the large-scale production of erythritol using this non-conventional yeast. The plan involved the disruption of anabolic pathways for the byproducts and catabolic pathways for erythritol, along with overexpression of the RSP5 gene encoding ubiquitin ligase from *S. cerevisiae*; this resulted in the creation of a modified strain of *Y. lipolytica* with enhanced heat resistance, elevated NADPH production, and diminished production of mannitol byproduct, thereby enhancing the overall yield of erythritol to 196 g/L in 22,500 L fermenter in 78 h. This development is pivotal for process optimization, offering potential cost reductions associated with bioreactor cooling and the purification of erythritol (Wang et al., 2020a).

5.3.2. *p-Coumaric acid*

p-Coumaric acid (4-hydroxycinnamic acid; p-CA) is a phenolic acid that is biologically synthesized through the shikimate pathway (Pei et al., 2016). *p*-CA serves as an important precursor for the synthesis of many flavonoids and is of interest as an active ingredient in cosmetics due to its antioxidant, anti-inflammatory, and antimutagenic properties (Boo, 2019; Zhu et al., 2024). Despite its availability in plants, the extraction of *p*-CA from plants is costly and inefficient due to limited plant resources. Compared to chemical synthesis processes, biosynthesis of *p*-CA can offer a cheaper and more environmentally sustainable route (Zhu et al., 2024). Zhu et al. engineered *Y. lipolytica* for effective biosynthesis of *p*-CA by integrating multiple copies of the TAL gene encoding tyrosine ammonia lyase from *Rhodotorula glutinis*, directing metabolic flux towards *L*-tyrosine (*L*-Tyr) synthesis and blocking competitive pathways to *L*-tryptophan (*L*-Trp) and *L*-phenylalanine (*L*-Phe) production (Zhu et al., 2024). Cellulose and hemicellulose hydrolases were then introduced to enable the yeast strain to utilize cellulose and hemicellulose as the sole carbon source. Using carboxymethylcellulose and xylan from bagasse as representative substrates of cellulose and hemicellulose, the engineered strain produced *p*-CA titers of 84.3 mg/L and 65.3 mg/L, respectively, in 50-mL cultivations within 5 days, demonstrating the potential of producing the bioproducts from lignocellulose biomass. However, these production titers were noted to be significantly lower compared to the *p*-CA titer produced under the same culture conditions using glucose as the substrate (1.035 g/L). As a follow-up, the authors propose implementing ALE on the engineered *Y. lipolytica* strain, with cellulose and xylan as carbon sources, to improve the utilization of these substrates.

In 2025, Jin Hou's group also incorporated prenyltransferase (AcPT1) gene from *Artemisia capillaris* to further convert *p*-CA into drupanin, a. bioactive compound which has a tremendous potential as anti-cancer drugs (Liu et al., 2025). As their best results, 52.26 mg/L of product was obtained *via* fed-batch fermentation in 3 L bioreactor.

5.3.3. *Cordycepin*

Cordycepin (3′-deoxyadenosine) is a nucleoside compound derived from natural microorganisms, particularly *Cordyceps militaris*. In 2022, the market value of this bioactive compound nearly reached $440,000/kg due to its diverse nutraceutical properties associated with anti-inflammation and anti-ageing (Duan et al., 2023b). Microbial synthesis presents an alternative approach to mitigate the decline of biodiversity caused by the heightened extraction of cordycepin from wild resources. Recently, *Y. lipolytica* has been demonstrated as a promising chassis for cordycepin production from agro-industrial residues such as sugarcane molasses, waste spent yeast, and diammonium hydrogen phosphate, with a product titer of 2.3 g/L achieved in 120 h in a shake flask containing 50 mL of optimized media. The engineered strain was produced through overexpression of the genes CNS1, CNS2, GLK1, and PGM1 encoding oxidoreductase / dehydrogenase enzyme complexes, metal-dependent phosphohydrolases, glucokinase, and phosphoglucomutases, respectively, to optimize hexose utilization and carbon flux in the glycolysis and PPP pathways (Duan et al., 2023a).

5.3.4. *Gastrodin*

Gastrodin (hydroxybenzyl alcohol-4-O-β-D-glucopyranoside) is a phenyl glycoside known for its neuroprotective properties, which make it beneficial for treating headaches, dizziness, epilepsy, and neurodegenerative conditions (He et al., 2021; Wu et al., 2024). Metabolic engineering of the PPP in *Y. lipolytica* offers a sustainable and scalable method for gastrodin production. Drawing insights from gastrodin production using metabolically engineered *E. coli*, *S. cerevisiae*, *Bacillus subtilis*, *Rhodiola glycosyltransferase*, and *Nocardia sp.* cells, Wu et al. integrated a heterologous gastrodin biosynthetic pathway into the *Y. lipolytica* genome (Wu et al., 2024). Further improvements in gastrodin production yield were achieved through overexpression of the shikimate pathway and alleviation of competitive pathways. After 132 h, a gastrodin titer of 13.2 g/L in a 2-L fed-batch fermenter represented the highest reported level of microbially produced gastrodin.

5.3.5. *Resveratrol*

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), widely marketed as a dietary supplement and cosmetic ingredient, is expected to witness an annual market value growth rate of 8% from $97.7 million between 2018 and 2028 (Wang et al., 2020c). This compound is a secondary metabolite produced in plants using the aromatic amino acids *L*-Phe or *L*-Tyr as precursors in the biosynthesis process (Liu et al., 2022a). Although early attempts at resveratrol production in *Y. lipolytica* yielded low titers in the mg/L range (Wang et al., 2020c), continuous efforts in metabolic engineering of the shikimic acid pathway have resulted in huge improvements in production titers, with up to 22.5 g/L reported in recent work (Liu et al., 2022a). This noteworthy progress in resveratrol production was achieved by boosting precursor molecules, fine-tuning carbon metabolism, amplifying essential pathway genes through randomized multi-copy genomic integration followed by high throughput screening, and precisely controlling *Y. lipolytica* cells to maintain the yeast form throughout a meticulously optimized fed-batch fermentation process (Liu et al., 2022a).

5.3.6. Scutellarin

Scutellarin (4,5,6-trihydroxyflavone-7-glucuronide) is a bioactive compound originating from *Erigeron breviscapus*, a herb widely used in traditional Chinese medicine (Zhang et al., 2023). The market demand for scutellarin has been steadily increasing due to its potential therapeutic benefits, particularly in treating cerebral ischemia and Alzheimer's disease (Xie et al., 2024). However, the challenges associated with extracting scutellarin from the *E. breviscapus* plant and the environmental and cost issues linked to chemical synthesis have prompted the exploration of alternative production methods (Zhang et al., 2023).

Notably, a recent study employed a structured metabolic engineering approach to produce scutellarin in *Y. lipolytica*, which involved: (i) enhancing the combination of flavone-6-hydroxylase-cytochrome P450 reductase (SbF6H-ATR2) to enhance P450 enzyme activity, (ii) boosting the expression of crucial enzyme genes, (iii) upregulating 6-phosphoglucose dehydrogenase (ZWF1) and 6-phosphoglucose dehydrogenase (GND1) genes to replenish NADPH levels, (iv) augmenting the availability of precursors such as *p*-coumaric acid and uridine diphosphate glucose, and (v) improving oxygen availability through the expression of bacterial hemoglobin (Zhang et al., 2023). The systematic metabolic engineering strategy resulted in a production titer of 0.703 g/L of scutellarin in a 50-mL shake flask fermentation in 84 h, marking the highest titer reported to date.

5.3.7. Dihydroquercetin

Dihydroquercetin (DHQ) is a dihydroflavonol compound which is widely used as dietary supplements and Active Pharmaceutical Ingredients (API) in attribution to its antioxidant, anti-cancer, anti-bacterial, and anti-inflammatory properties (Terekhov et al., 2024). While its global demand keeps increasing, the availability of this compound is very limited and its content in plant is only 3% (Yu et al., 2021). Therefore, advanced biological approach for the production of DHQ is highly preferred.

The *de-novo* production of DHQ in *Y. lipolytica* could be done by further extending the p-coumaric acid pathway within the PPP pathway. Firstly, p-coumaric acid is converted into (2*S*)-naringenin (NAR) which are catalyzed by 4CL, CHS, and CHI encoding 4-coumaryl ligase, chalcone synthase, and chalcone isomerase, respectively. Subsequently, the conversion of NAR to the intermediate (2*S*)-eriodictyol and the DHQ product was done by further incorporation of flavonoid-3-hydroxylases (F3H) (Li et al., 2024; Lv et al., 2019).

The first bioproduction of DHQ in *Y. lipolytica* was previously reported in 2019 by Lv., et al. 110.5 mg/L of product was successfully obtained by overexpressing and increasing the copy number of CHS and CPR gene encoding cytochrome P450 reductase (Lv et al., 2019). Despite this success, the synthesis of DHQ is still hindered by the lack of ERI supply and the low of activity of the substantial enzyme F3H.

Recently, *Y. lipolytica* YE54, a high producing-ERI strain was used as a parental strain to construct a novel DHQ-producing strain through the engineering of NADPH regeneration pathway, optimization of F3H, together with the increment of oxygen and 2-oxoglutarate supply. In the end of the day, 181.4 mg/L of DHQ was obtained in the shaking flask (He et al., 2025). System scale-up in 5 L fed-batch bioreactor further gave 4.2 g/L of product, highlighting it as the highest bioproduction of DHQ that has been reported so far.

5.3.8. Sakuranetin

Sakuranetin is a flavonoid phytoalexin molecule which has been extensively utilized in cosmetics and pharmaceuticals in attribution to its antiviral, antifungal, and anti-inflammatory physiological properties (Chen et al., 2023; Ullah et al., 2020). Although it is abundantly available in nature (*e.g.* barks, leaves, *etc.*), its chemical extraction processes remain challenging and inefficient. Biologically-produced sakuranetin is also still hindered by the low production yield (Wang et al., 2020b). Therefore, substantial metabolic engineering strategies remain for further investigation to boost up the production of this compound.

In contrast to DHQ biosynthesis, sakuranetin production in *Y. lipolytica* also diverges from the shared p-coumaric acid metabolic flux, specifically at (2*S*)-naringenin (NAR) node. After 4-CL, CHS, and CHI catalyze the formation of NAR, the pathway is redirected towards sakuranetin *via* regio-specific methylation through the incorporation of NOMT gene encoding Naringenin-*O*-methyltransferase.

The use of *Y. lipolytica* for the production of sakuranetin was firstly reported by Yang Gu's research group back in 2024. In this study, a multimodule engineering strategy was implemented: sakuranetin biosynthesis pathway optimized, malonyl Co-A supply was enhanced, regeneration system for the methyl donor S-adenosyl methionine was also introduced. Under this condition, the engineered strain could successfully produce the target product titer of 344 mg/L in a 5 L bioreactor (Ge et al., 2024). This demonstrates that Yarrowia could be used as a promising host for sakuranetin biosynthesis.

More recently, a similar strategy was also introduced by Xu, et al. in 2025. Additionally, the group also performed a promoter screening to increase the methyltransferase expression level, boosted up the Shikimate pathway metabolic flux, and adjusted the substrate (glucose) concentration (Xu et al., 2025). As a result, 687 mg/L of sakuranetin was produced at the end of 7 days of fermentation, representing the best product titer that has been reported to date.

5.3.9. Aromatic-derived natural products

The shikimate pathway plays a crucial role in the production of aromatic compounds by using E4P generated from carbon metabolism in the PPP to facilitate the biosynthesis of essential aromatic amino acids like phenylalanine, tyrosine, and tryptophan (Masi et al., 2021). These amino acids are vital for protein synthesis and act as precursors for a broad spectrum of aromatic-derived natural products, encompassing secondary metabolites, pigments, vitamins, and pharmaceuticals (Krivoruchko and Nielsen, 2015). The utilization of *Y. lipolytica* as a microbial chassis for *de novo* synthesis of various aromatic-derived natural products and chemicals was recently reported. Gu et al. adopted a systematic approach to address the rate-limiting steps within the shikimate pathway in *Y. lipolytica*, which involved optimizing amino acid formation and engineering feedback-insensitive 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthases. Additionally, metabolic activity was enhanced by overexpressing phosphoketolase and deleting pyruvate kinase, thereby creating a sustained metabolic driving force through the shikimate pathway. By effectively blocking competing precursor pathways and reducing byproduct formation, the engineered *Y. lipolytica* was able to synthesize aromatic compounds such as 2-phenylethanol (2-PE) and violacein using glucose as a substrate. The engineered strain produced significantly higher titers of these compounds compared to the initial strain, showcasing the potential of *Y. lipolytica* as a sustainable biorefinery chassis for aromatic compound synthesis (Gu et al., 2020).

A recent study achieved a similar yield of 2-PE by integrating

CRISPR-Cas9 and metabolic engineering, offering key insights into optimizing the *de novo* synthesis of this fragrance compound in *Y. lipolytica*. The introduction of two feedback-resistant enzymes from *E. coli*, EcAROG^{fbr} and EcPheA^{fbr}, into the yeast genome effectively reduced tyrosine-induced inhibition in the shikimate pathway, achieving a 2-PE production titer of 0.33 g/L after 60 h. Next, the Ehrlich pathway was optimized by co-expressing ARO10 and PAR4, addressing the rate-limiting step and increasing titers to 0.45 g/L. Further enhancement was achieved by increasing the copy numbers of the EcAROGf^{br}, EcPheA^{fbr}, ARO10, and proteinase-activated receptors (PAR4) genes and eliminating competing pathways through the knockout of prephenate dehydrogenase (TYR1), raising 2-PE titers to 1 g/L. Attempts to knock out anthranilate synthase (TRP2) and pyruvate kinase (PK) led to growth defects, underscoring the complexity of pathway balance. Overexpressing transketolase (TKT) and shikimate pathway enzymes ARO1 and prephenate dehydratase (PHA2) increased precursor availability, boosting production to 1.35 g/L. Finally, increasing the glucose concentration to 40 g/L further elevated 2-PE yields to 2.4 g/L after 84 h (Qian et al., 2024).

5.4. *Fatty acid biosynthesis*

5.4.1. *Unusual fatty acids*

Recently, unusual lipid production for *Yarrowia lipolytica* was reviewed (Park and Nicaud, 2020). In this article, we have tried to capture the more recent efforts. Conjugated fatty acids are an interesting group of fatty acid derivatives. They are useful as dietary supplements and nutritional lipids. Conjugated linoleic acid (CLA) has been shown to be effective against cancer, obesity, and atherosclerosis (Basak and Duttaroy, 2020). CLA has been successfully produced using *Yarrowia lipolytica* to a titer of 3.1 g/L in a bioreactor when cultivated in a medium supplemented with soyabean oil (Zhang et al., 2013). This was achieved through heterologous expression of the delta 12-desaturase gene (*FADS12*, *d12*) from *Mortierella alpina* and a linoleic acid isomerase gene from *Propionibacterium acnes* in *Yarrowia lipolytica* PO1h (Zhang et al., 2013). In another report, 15.6 g/L CLA was produced using permeabilized cells by freeze-thaw cycle and supplementing media with 25 g/L of LA and 1.5 g/L sodium acetate (Zhang et al., 2016). Glucose has also been used to produce CLA and mg/L range titres have been achieved (Imatoukene et al., 2020b; Wang et al., 2019).

Cyclopropane fatty acids are unusual fatty acids that are beneficial for resistance to oxidation and fluidity of lipids at low temperatures. Heterologous cyclopropane fatty acid synthases from various organisms have been expressed in *Yarrowia lipolytica* (Czerwiec et al., 2019; Markham and Alper, 2018) under various promoters, with the final enzyme from *E. coli* under the hpd8d promoter generating a titer of 2.3 g/L (Czerwiec et al., 2019). Co-overexpression of the LRO1 gene encoding acyltransferase has also been shown to improve the titer (Imatoukene et al., 2020a).

Punicic acid is an omega-5 PUFA with various nutraceutical benefits (Grossmann et al., 2010; Machado et al., 2022; Shabbir et al., 2017). Expression of the bifunctional fatty acid conjugase and desaturase from *Punica granatum* from a strong erythritol-inducible promoter produced 36.6 mg/L of punicic acid. Improving the levels of C18:2 linoleic acid (a precursor of Punicic acid) by overexpressing fatty acid desaturases (FAD2) enhanced punicic acid production. Overexpression of the CPT gene encoding carnitine palmitoyltransferase also increased the pool of oleic and linoleic acid entering PC (phosphatidylcholine), thus further improving punicic acid production (Wang et al., 2024b).

Nervonic acid is a long-chain mono-unsaturated fatty acid which benefits neurological health and has therapeutic applications (Vozella et al., 2017). Recently, multiple genetic engineering efforts have been reported to be involved in nervonic acid production. In the first report, a four-step genetic engineering strategy led to improved nervonic acid and overall lipid production. In the first step, β-ketoacyl-CoA synthase CgKCS, fatty acid elongase gELOVL6, and desaturase MaOLE2 were expressed, leading to an increase in nervonic acid titer. In the second step, glycerol-3-phosphate acyltransferases and diacylglycerol acyltransferases genes from *Malania oleifera* were overexpressed in the ER, resulting in improved overall lipid production. ER structure regulator gene YlINO2 was overexpressed in the third step, and SNF1 was deleted in the fourth step. SNF1 deletion led to an improved ratio of nervonic acid to lignoceric acid by 61.6%. Final strain had a nervonic acid titer of 17.3 g/L (17.9% of TFA) (Su et al., 2023). In another report, various β-ketoacyl-CoA synthases and heterologous Δ15 desaturases were co-expressed in *Yarrowia lipolytica*. This was combined with the deletion of the β-oxidation pathway to achieve a nervonic acid titer of 13.56 g/L. In this study, the native lysophosphatidic acid acyltransferase was swapped with the one from *Malania oleifera,* which is more specific to nervonic acid, thus incorporating this fatty acid into the TAG (K. Wang et al., 2023a). Another recent report showed that using an auxiliary carbon source like colleseed oil increased nervonic acid production as compared to glucose (Zhao et al., 2023).

Ricinoleic acid (RA) is a monounsaturated fatty acid with unique properties and is used as petrochemical replacement in various industrial processes. A recent study achieved a high titer (2.061 g/L) of RA in the form of free fatty acid through lipid flux redirection towards oleic acid heading to PC. Additionally, Triton X-100 was used to secrete RA from the cells and alleviate cell toxicity issues. *Claviceps purpurea* Δ12 oleate hydroxylase gene was overexpressed, and multiple genes (MEF1, PEX10, FAD2, PAH1, APP1 and DGA1) were deleted to redirect the flux of oleic acid towards PC. PC biosynthesis was also boosted through overexpression of genes PSD1, CDS1, OPI3 and CHO2 (Park and Hahn, 2024). Previous efforts in this space (Beopoulos et al., 2014; Guo et al., 2018) had led to a titer of 2.2 g/L RA when *Y. lipolytica* was grown on cellulose as a C-source (Guo et al., 2018).

5.4.2. *Fatty acids with varying chain lengths*

Medium-chain fatty acids (MCFA) are of interest in biofuel applications. MCFA was successfully produced in *Y. lipolytica* by expression of thioesterases with specificity towards medium-chain acyl-ACP. MCFAs were incorporated into various classes of lipids and majorly comprised of C10 and C8 (Rutter et al., 2015). Other genes of the FAS system have also been engineered to modulate the specificity of fatty acid produced. For example, ketoacyl synthase (KS) was engineered to modify its substrate binding pocket, leading to a significant increase in the production of C14 fatty acid (Rigouin et al., 2017). Overexpressing a plant DGAT encoding diacylglycerol acyltransferases specific to MCFA also improved cell growth, demonstrating further how lower amounts of essential fatty acids could be compensated (Rigouin et al., 2018). Another study reported truncating native FAS1 encoding fatty acid synthetase and fusing the truncated version with thioesterases specific to medium-chain acyl-ACP. This led to a remarkable increase in C12 and C14 fatty acid production (Xu et al., 2016).

Very long-chain fatty acids (VLCFA) are industrially attractive molecules. VLCFA, like behenic acid (C22:0) and erucic acid (C22:1Δ13), are generally derived from plant oils. VLCFA production was achieved by overexpressing native DGA1 and a 3-ketoacyl-CoA synthase (FAE1 from *Thlaspi arvense*) and deleting the MFE1 gene from the β-oxidation pathway. The best strain from this study achieved a VLCFA yield of 120 μg/g DCW (34% of TFA) (Gajdoš et al., 2022). Alternative carbon sources like glycerol and waste cooking oil have also been used to grow genetically engineered *Y. lipolytica* strains (ΔFAD2 and overexpression TaFAE1) to produce erucic acid (at 887 mg/L yield) (Gajdoš et al., 2020). Another strategy employed to improve VLCFA production is overexpressing KCS from *Arabidopsis thaliana* and inhibiting the beta-oxidation pathway by deleting the PEX10 gene (Gao et al., 2020).

Odd chain fatty acids (OCFA) have a range of applications in nutrition (Jenkins et al., 2015) to therapeutics (Degwert and Jacob, 1998). In one of the first reports of OCFA production, the PHD1 gene was deleted in a superior lipid-producing strain (Table 8). Using a fed-batch process and co-feeding of glycerol and propionate, this strain produced 0.75 g/L of OCFA (Park et al., 2018). Later, the same group came up with a genetic engineering strategy to produce OCFA without propionate supplementation at 0.36 g/L titer. Seven genes were expressed in high lipid-producing *Y. lipolytica* strain to convert oxaloacetate to propionyl-CoA (Park et al., 2020). A more recent effort reports overexpressing propionate activating enzymes and balancing the ratio of acetyl-CoA and propionyl-CoA precursor pool. For the OCFA initiation step, higher amounts of propionyl-CoA is required. Overexpressing propionate activating enzyme led to increase in the propionyl-CoA precursor pool. Since acetyl-Coa is required for the elongation steps for, hence removing acetyl-CoA completely is not feasible. Therefore, the ratio of acetyl-CoA and propionyl-CoA was optimized. Combining this with C/N optimization, OCFA titer was improved up to 1.87 g/L (Park et al., 2021).

5.4.3. Lipid product mimics

Y. lipolytica has been used to produce various product mimics like cocoa butter, human milk fat, high oleic oils, *etc.* (Table 8). Papanikolaou et al. produced cocoa butter equivalent (CBE) at 3.4 g/L by growing cells in a mixture of agro-industrial residue containing a derivative of stearin, technical glycerol (a bio-diesel byproduct) and glucose (Papanikolaou et al., 2003). Optimization of fermentation conditions like media composition and temperature control have also been used to achieve cocoa butter-like composition (Zhao et al., 2016). A targeted genetic engineering approach has also been used to generate CBE more recently. C16:1 was abolished by changing the OLE1 gene to a homolog from *Rhodotorula toruloides*. C18:2 amounts were reduced by promoter modification or deletion of the FAD2 gene (Konzock et al., 2022). Human milk fat has a specific stereoisomeric structure where it has C16:0 palmitic acid at the *sn-2* position while unsaturated fatty acids at *sn-1/3*. Bhutada et al. expressed lysophosphatidic acid acyltransferase with specificity to C16:0 in *Y. lipolytica* and cultured it on palm oil to obtain TAG composition similar to human milk fat (Bhutada et al., 2022). High oleic oils are of interest due to their application as lubricants and in producing alternatives to high oleic plant oils like soybean and sunflower oil. By deleting the beta-oxidation genes, overexpressing lipid synthesis gene and deleting genes like FAD2, which convert oleic acid to PUFA, Wang et al. were able to generate a *Y. lipolytica* strain capable of 56 g/L lipids with 84% oleic acid content (Wang et al., 2022a, 2022b). A second report took another approach to genetic engineering by changing OLE1, GPAT and DGAT to homologous genes and overexpressing ELO2 (Tsakraklides et al., 2018).

5.4.4. Fatty alcohols

Fatty alcohols are a range of industrially important oleochemicals that are currently derived from petrochemicals. *Yarrowia lipolytica* has been extensively used to produce fatty alcohols (Table 8). One of the first reports was in 2016 when Wang et al. targeted genetic engineering by deleting genes in the β-oxidation pathway, TAG biosynthetic genes like DGA and LRO and sterol synthesis pathway genes like sterol acyltransferase (ARE1). This would channel available fatty acyl CoA into the fatty alcohol production pathways. They introduced a fatty acyl-CoA reductase (FAR) for the formation of alcohols and further deleted

Table 8
Bioproducts derived from the fatty acid biosynthesis pathway.

Product	Substrate	Engineering Strategy	Titer (g/L)	Yield (g/g substrate)	Scale	Time (h)	References
Punicic acid	Glucose	PgFADX↑, FAD2↑, CPT↑, PgLPCAT↑, LRO1↑, ΔPEX10, GPD1↑	3.07	0.012	Fed-batch bioreactor (3 L)	84	(Wang et al., 2024b)
Fatty alcohols	Glucose	MhFAR↑	5.8	0.036	Fed-batch bioreactor (1.7 L)	240	(Cordova et al., 2020)
Triacylglycerol (human milk fat substitute)	Glucose supplemented with palm oil	ΔGSY1, TEF1↑, LPAAT2↑, URA3↑	3.35	0.186	Bioreactor (0.5 L)	96	(Bhutada et al., 2022)
Cyclopropane fatty acids	Glucose	EcCFA↑	2.3	0.056	Fed-batch bioreactor (3.5 L)	72	(Czerwiec et al., 2019)
Cyclopropane fatty acids	Glucose	ΔPEX10, ΔMFE1, DGA1↑, ycoCFA↑	3.03	0.020	Pulse-fed bioreactor (1.7 L)	168	(Markham and Alper, 2018)
Omega-6 PUFAs (DGLA, GLA, and ARA)	Glucose	FAD2↑, IgΔ9E, IgΔ8D, CPT↑, ΔPEX10, ACC1↑ (DGLA) FAD2↑, MaΔ6D, CPT↑, ΔPEX10, ACC1↑ (GLA) ΔPEX10, ACC1↑ (ARA)	0.39 (DGLA) 0.83 (GLA) 0.19 (ARA)	0.0065 (DGLA), 0.014 (GLA), 0.0032 (ARA)	Flask (50 mL)	120	(Wang et al., 2023)
Ricinoleic acid	Glucose	ΔMHY1, CpFAH12↑, MaC16E↑, TRP1↑, CHO1↑, PSD1↑, CHO2↑, OPI3↑, CDS1↑, ΔPAH1, ΔAPP1, ΔTRP1, ΔURA3, ΔC3, CpFAH12↑, LEU2, ΔPEX10, ΔDGA1, ΔMFE1, ΔFAD2, ΔKU70	2.061	0.05	Flask (10 mL)	120	(Park and Hahn, 2024)
Oleic acid	Glucose	ΔGUT2, GPD1↑, ΔTGL4, ΔPEX10, ΔINTC, ACC1↑, DGA1↑, ΔSCP2, MaELO2↑, ELO1↑, ΔFAD2, ΔLIP1, PgSCD↑, ΔSCD, A08::LEU2↑	46.23	NA	Fed-batch bioreactor (3 L)	120	(Wang et al., 2022a)
Conjugated linoleic acid	Soybean oil	MaFADS12↑, OPAI↑	3.1	0.155	Bioreactor (3 L)	38.5	(Zhang et al., 2013)
Eicosapentaenoic acid (EPA)	Glucose	ScG6PD↑, ScACS↑, ScACC↑, ScPDC↑	0.27	0.0045	Flask (50 mL)	96	(Jia et al., 2024)
Docosahexaenoic acid (DHA)	Glycerol	Pfa1↑, Pfa2↑, Pfa3↑, AfPPT↑	1.04	0.0046	Fed-batch bioreactor (0.5 L)	144	(Jovanovic Gasovic et al., 2023)
Nervonic acid	Glucose	CgKCS↑, gELOVL6↑, MaOLE2↑, MoDGAT2↑, MoGPAT↑, INO2↑, ΔSNF1	17.3	0.006	Fed-batch bioreactor (50 L)	216	(Su et al., 2023)
Nervonic acid	Glucose	ΔTGL4, ΔGUT2, GPD1↑, ΔRAS2, McE2↑	13.56	NA	Fed-batch bioreactor (3 L)	168	(Wang et al., 2023a)

genes for the alcohol oxidative degradation pathway. They further overexpressed genes like ACL1, ACC1, ACS, and fatty acyl-CoA synthetase (FAA) to pool more carbon into the fatty acid biosynthesis pathway. The strains thus generated produced 636.89 mg/L intracellular hexadecanol (Wang et al., 2016b). Another study in the same year reported 167 mg/L production of hexa- and octadecanol by expressing FAR encoding fatty acyl-CoA reductase from *Marinobactor aquaeolei* VT8 (Wang et al., 2016a). Promoter screening led to the identification of promoter of PFBAin, which, when used to express FAR, led to improved production. Coupling this with further engineering of regulatory genes of lipid metabolism and optimization of fermentation conditions, Zhang et al. improved the fatty alcohol titer to 5.75 g/L (Zhang et al., 2019a). More recent reports have used similar strategies, such as expressing homologous FAR enzymes, using a push-and-pull strategy for overall lipid accumulation, and using fed-batch cultivation to improve fatty alcohol production (Cordova et al., 2020; Hambalko et al., 2021).

5.4.5. *Omega 3 and Omega 6 PUFA*

PUFA production in *Yarrowia lipolytica* was recently reviewed (Jia et al., 2022). Hence, only recent PUFA production efforts have been reviewed in this section (Table 8). Dietrich et al. expressed four cluster genes (pfa1, pfa2, pfa3, ppt) from myxobacteria under a strong minLEU2 promoter with upstream activating four UAS1B4 sequences to produce DHA. A 5’ TEF intron was also added to improve DHA production by 4-fold. Interestingly, they found that a smaller pool of acetyl-CoA leads to better production of DHA. Overall, 16-fold improvement was achieved using the engineering strategies mentioned earlier (Dietrich et al., 2023). Gasovic et al. employed a multi-omics approach by combining metabolomics, transcriptomics, and 13C-based metabolic pathway profiling to identify bottlenecks in DHA production during the metabolic switch from growth to stationary phase PUFA production. They improved DHA production by 40% by supplementing with small amounts of amino acid L-lysine. The key finding that metabolically, the drop in acetyl-CoA and malonyl-CoA pools at the start of DHA production is supplemented by the breakdown of amino acids like L-lysine, L-leucine and L-isoleucine could have a significant impact in further engineering for production of various products in *Y. lipolytica* (Jovanovic Gasovic et al., 2023). For EPA production, the acyl-CoA: lysophosphatidylcholine acyltransferase from diatom *Phaeodactylum tricornutum* (PtLPCAT1) was shown to improve EPA production more than 2 folds. This report shows that the overexpression of this gene improves EPA production by exchange of D9-elongation product (C20:3, D11,14,17) intermediate between phospholipids and acyl-CoA pool (Katiyar and Arora, 2020). EPA production at more than 15% of DCW has been achieved using *Y. lipolytica via* the Δ9 elongase pathway and was also scaled for commercial production. Pex10 gene deletion played a crucial role in achieving high levels of the EPA (Xie et al., 2015; Xue et al., 2013). Higher titres of EPA were achieved recently by using *Y. lipolytica* that can grow efficiently on waste cooking oil and deletion of TAG lipases, so that the carbon absorbed can be channelled into EPA production (Qin et al., 2025).

Elevated incorporation of EPA into TAGs was attempted by Jia et al. through the expression of DGAT, GPAT, ACC, ACS, G6PD, and pyruvate dehydrogenase complex (PDC) from *Schizochytrium sp.* In addition, DGAT protein engineering was performed to replace the HFS motif in DGAT2A with the motif YFP. With these engineering strategies, a titer of 266.4 mg/L was achieved (Jia et al., 2024). Wang et al. also engineered multiple strains to produce omega-6 PUFAs dihomo-γ-linolenic acid (DGLA), γ-linolenic acid (GLA), and arachidonic acid (ARA) up to a titer of 832.00, 386.59 and 191.76 mg/L, respectively. Δ6 (from *Mortierella alpina)* and Δ8 (from *Isochrysis galbana*) pathways were assembled for the production of ARA. To further optimize the production, the PC pool was enriched for ARA by converting the oleic acid at the *sn-2* position to an ARA (Wang et al., 2023).

6. Fermentation technology

The development of fermentation technology in *Yarrowia lipolytica* has become increasingly attractive and encouraging to establish a sustainable platform to produce bio-based chemicals and high value products. Due to its physical characteristic, high metabolic versatility, and excellent tolerance to environmental stresses, this non-conventional yeast is superior in several aspects over conventional microbial hosts for industrial fermentation. It can efficiently utilize various economic and easily available renewable substrates, which renders it as a promising organism for cost-effective and environmentally friendly process development. Recent technological advancements such as continuous fermentation, perfusion fermentation with high cell density, as well as AI-assisted fermentation process monitoring and control contribute a major role in fermentative bioproduction of high-value biochemicals.

Over the past decade, the continuous fermentation in *Yarrowia lipolytica* has emerged as a transformative strategy for industrial biotechnology, enabling stable product formation over extended periods of time while maintaining stable bioprocess performance (Kamzolova, 2024). In addition, continuous operation will also reduce downtime, minimize labor work, and increase volumetric productivity, making it highly favorable for large-scale bioprocessing of organic acids, lipids, and other high-value biochemicals (Xie, 2022). Quinn Zhu's research group was among the first to report the use of continuous fermentation of *Y. lipolytica* Z7334 for producing Omega-3. A two-stage continuous fermentation was established, consisting of a smaller growth fermenter as the first stage, followed by the larger production fermenter. This approach successfully outperformed the conventional 2 L fed-batch method by boosting overall the Omega-3 productivity by 80% and product concentration by 40%, while maintaining equivalent the product yield from glucose (Xie et al., 2017).

Recently, a β-carotene-producing *Y. lipolytica* strain was also investigated under the continuous fermentation approach. Surprisingly, it was found that continuous bioreactor operation led to a significantly lower product titer - giving only 18 growth generations, which was almost half than that of 30 growth generations obtained from the shaking flask fermentation (Worland et al., 2025). This phenomenon occurred in attribution to the limited oxygen transfer and the fermenter high dilution rates. The study further revealed that the selection of carbon source will also affect the entire process, with canola oil helping to boost up and prolong the β-carotene synthesis. At the end of the day, the findings emphasize that continuous fermentation does not always guarantee high product titer. The actual outcome will still highly depend on the strain characteristics, carbon sources, cultivation modes, and other process parameters.

Perfusion fermentation enabling high cell density cultures in *Yarrowia lipolytica* represents a major advancement in maximizing productivity and metabolic output in industrial bioprocesses. By continuously supplying fresh medium while simultaneously removing spent culture and inhibitory by-products, perfusion systems maintain cells in an optimal physiological state, enabling exceptionally high biomass levels that are difficult to achieve in conventional batch or fed-batch systems. This approach was also studied by Dongming Xie's group in 2025 using *Y. lipolytica* Po1f (Dpex10Dmfe_Leu + Ura + DGA1) to enhance to production of lipid. The work uses a model-guided continuous fermentation combined with adaptive laboratory evolution (ALE) approach to substantially improve the lipid production with the engineered strain (Kurt et al., 2026). As their best results, 150 g/L of biomass was successfully produced within 47 days in 1 L bioreactor. It was also observed that lipid productivity was significantly enhanced from 0.3 to 0.5 g/L/h (*via* fed-batch fermentation) to ~1 g/L/h, with the product yield remains consistent at 1 g/g substrate. This strategy serves as one of the promising and effective methods to produce microbial lipids at commercial scales with high productivity.

The implementation of AI-assisted fermentation process monitoring and control systems in *Yarrowia lipolytica* has also emerged as a vital approach to enhance bioprocess optimization through continuous real-time prediction, process adjustment, and decision-making during cultivation. Machine learning analyze process fermentation data continuously to forecast process variations which enables them to control different bioprocess parameters. For instance, integrated knowledge mining, genome-scale modelling (GSM), and machine learning for predicting *Y. lipolytica* bioproduction was reported by Czajka et al. (2021). The work comprises data-driven model to predict how well this strain would perform as a host across many precursors, products, strains, and process conditions to produce these metabolites during the fermentation. By manually compiling data from over 100 publications and applying GSM and flux balance analysis (FBA), it was identified that metabolic profile from FBA was one of the most critical factors, alongside with substrate inputs, number of enzymatic steps, as well as the thermodynamic barriers (Czajka et al., 2021). The authors also show that this integrated study can be extended to other type of yeasts and served as a platform for predicting metabolic and enzyme engineering target molecules.

Coşgun et al. (2022) similarly applied a machine learning algorithm to analyze lipid production from this exceptional strain. A huge dataset consists of 356 entries from various experimental results was used to classify the key elements (*i.e.* pH, aeration, C/N ratio, fermentation time, *etc.*) that drive a pivotal role to give the highest lipid profile and product titer across diverse substrates (Coşgun et al., 2022). Overall, the study concludes that ML is highly effective for predicting and designing processes to maximize biomass growth and lipid yields. In the same year, Xiao-Jun Ji's team used an Artificial Neural Network (ANN)-based soft sensor model to overcome challenges in real-time monitoring of lipid fermentation (Wang et al., 2023b). Four variables (DO, initial glucose concentration, DO, and NaOH volume) was selected to predict the amount of cell dry weight, glucose, and lipid produced. This approach could also be implied to the other similar fermentation processes which depend heavily on pH and DO control. More recently, Wu et al. (2025) introduced a machine learning-assisted pipeline to design and evolve superior signal peptides (SPs) for enhancing heterologous protein secretion in *Y. lipolytica.* The work demonstrates that ML could effectively classify SP performance and enable computational screening and refinement of SP sequences, thus improving the success rate of protein secretion engineering (Wu et al., 2025). In conclusion, the implementation of AI and machine learning represents a major technological advancement that enhances process stability, accelerates commercialization, and strengthen the use of *Y. lipolytica* as a microbial host. These developments position this yeast as a strong contender for the future of industrial biotechnology.

7. Conclusion and future challenges

Yarrowia lipolytica emerges as an efficient and promising microorganism in various biotechnological applications. The advancement of *Y. lipolytica*-based strategies in metabolic engineering represents economic and environmental advantages over traditional physical and chemical treatments. Moreover, this non-conventional yeast harnesses unique metabolic pathways enabling the production of high-value biochemicals *via* its TCA, mevalonate, as well as fatty acid biosynthesis pathways. Several *Yarrowia* strains have been used in bioindustrial sectors, demonstrating the vast potential of application to this fungus.

With its exceptional characteristics, robustness, and recent progress in metabolic engineering, *Y. lipolytica* could serve as a frontrunner in shaping the future of biotechnology. As more industries and researches strive more towards sustainability, this cell line will also offer a huge opportunity for innovative solutions in waste valorization, bioremediation, sustainable high-value biochemical production, and biopharmaceutical manufacturing. This includes, but not limited to its application in machine learning, bioprocess engineering, and adaptive laboratory evolution (Fig. 4).

The use of Artificial Intelligence (AI) has been rapidly expanding in different types of chemical and biotechnological applications. To date, significant advancements have been achieved in the rational design of different cell factories using predictions and simulations *via* machine learning and genome-scale metabolic models. However, only a few of them focus on *Y. lipolytica*. The future of AI in building *Y. lipolytica* cell factories hold tremendous prospects and potential for revolutionizing biotechnological processes to another level.

There are different levels on how AI will significantly impact precision fermentation. The first stage is the use of machine learning for the identification of pathway construction and enzyme discovery and engineering. These objectives will help identify gene targets that can be engineered and constructed within the yeast complex metabolic networks, which subsequently leads to faster development of novel yeast variants. For instance, the combination use of computational tools BNICE.ch and BridgeIT helps to determine the pathway and necessary enzymes that could catalyze the target reaction (Hafner et al., 2021). The pinpointing of key regulatory enzymes could significantly improve predictions and simulations, thus reducing the need for extensive trial-and-error experiments.

Secondly, the use of machine learning for metabolic flux optimization can be used to predict how changes in gene expression, enzyme activity, or environmental conditions will affect the flow of metabolites through different pathways. By accurately predicting these interactions, machine learning helps in identifying the optimal conditions for directing metabolic flux towards the production of target compounds. Recently, a prosperous modelling approach has been established to control and predict the metabolic switch between glucose and ethanol fluxes in *Saccharomyces cerevisiae via* MATLAB using the Event Driven Method (EDM) and Time Stepping Method (TSM) (Jouned et al., 2022). As a result, the *S. cerevisiae* growth model could be adapted to multi-substrate mixtures by considering potential interactions where both ethanol and glucose fluxes are directly controlled to trigger switches between different metabolic pathways. Since *Saccharomyces sp.* and *Yarowia sp.* are considered budding yeast with many similarities, these technologies mentioned above could also be applied to *Yarrowia* strains.

AI-driven metabolic modelling and evolution strategies could also be applied to design and optimize bioprocess and downstream platforms on the industrial scale, especially in process monitoring with quality by design (QbD) and process analytical technology (PAT) applications. For instance, Pfizer has established a system wherein they used a total of 180 micro-bioreactors to assess scaled-down parameters compared to conventional 3-L bioreactors. Additionally, other AI tools like SIMCA from Sartorius were launched as hybrid modelling software, which enables statistical analysis, chemometrics, biomanufacturing, and process monitoring. The AI spectroscopy model by ThermoFisher Scientific also emerged as one of the technologies used to visualize and predict spectroscopy data. While bigger size and higher prediction accuracy are required, most industrial strains still lack several modelling key parameters (*e.g.*, the enzyme's Km and kcat), which hinders the broader application of AIs in industrial biotechnology. Therefore, further improvement is necessary to overcome this issue.

On the other hand, the use of alternative carbon and nitrogen sources for the growth of *Y. lipolytica* could also further contribute towards sustainability and a circular economy. Utilizing alternative carbon and nitrogen sources in the fermentation of *Y. lipolytica* offers significant potential for enhancing the sustainability of industrial bioprocesses. This approach not only reduces reliance on traditional and costly substrates but also contributes to the circular economy by utilizing waste material.

Recently, the use of waste cooking oil and cocoa fatty acid distillate as the main carbon sources for the fermentation of *Y. lipolytica* was reported (Rong et al., 2022; Sofeo et al., 2024). Since this cell line could utilize a diverse type of carbon source, including those derived from industrial and agricultural waste, it would be a great approach to extending the variety of side streams that can be used to grow this strain.

Utilizing *Yarrowia lipolytica* for low-water growth, sucrose secretion,

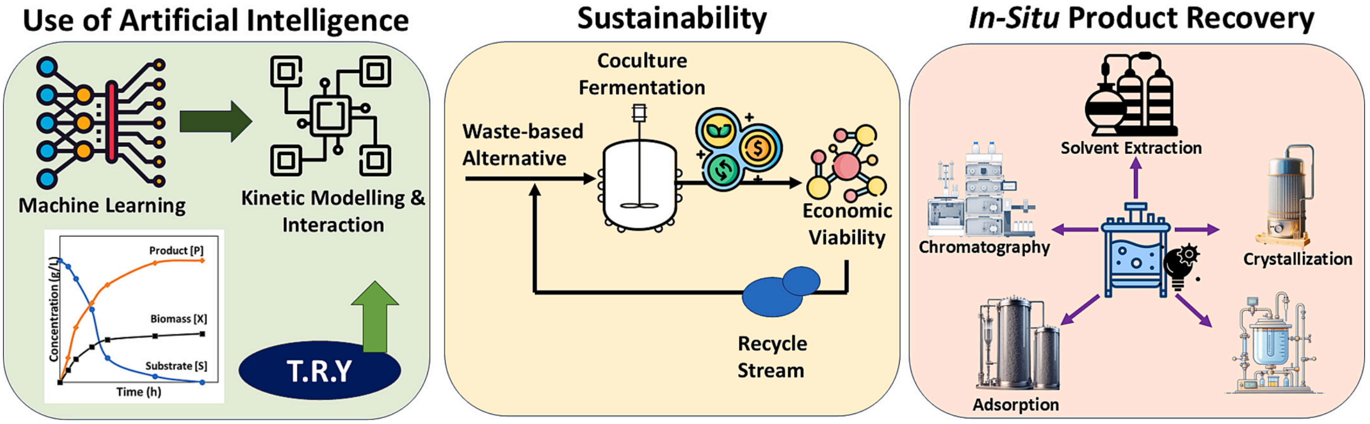


Fig. 4. Innovations that may improve industrialization of *Yarrowia lipolytica*-derived high value products.

and adaptive evolution for salt tolerance can enhance bioprocess efficiency. Recently, co-culturing sucrose-secreting Synechococcus *sp.* PCC7002 with *Y. lipolytica* enabled the production of β-caryophyllene, a molecule which is widely used in cosmetics and pharmaceuticals (Chen et al., 2024). This approach offers a promising alternative for sustainably producing high-value biochemicals *via* co-culture systems. A spontaneous syntrophic intra- and interspecies synthetic yeast community comprising six different auxotrophs of *Y. lipolytica* and *S. cerevisiae* was also developed to improve the bioproduction of 3-hydroxypropionic acid (3-HP). It was later found out that the co-culture of *Y. Lipolytica* △*trp4*-P and △*trp2*-B successfully give 4.67 mM of 3-HP, 19.3 times much higher than that of the 0.238 mM produced by the wild type strain coexpressing the 3-HP biosynthetic pathway (Park et al., 2024). This will definitely give out more alternatives for bioproduction of other high-value chemicals *via* co-culturing two or more different species of yeasts.

The future of *Y. lipolytica* in bioprocess and downstream processing areas is overwhelmed with promising potential areas of development. Innovations in downstream processing techniques, for instance, will play a pivotal role in maximizing the recovery and purification of desired products from *Y. lipolytica* cultures. Future developments may include the implementation of novel separation methods, such as membrane technologies or chromatographic approaches, integrated fermentation with crystallization, extractive distillation, simultaneous fermentation with *in-situ* product recovery, and hollow fibre adsorption to streamline purification processes and reduce overall production costs.

Despite these promising prospects, several challenges remain to fully commercialize *Y. lipolytica* in the biomanufacturing industries. Firstly, the formation of byproducts is one of the major problems to fully scale-up and commercialize the fermentation system. This occurred due to the significant carbon loss from the synthesis of product of interest, which leads to the formation of other organic acids, fatty acid, and other molecules that competes with the desired product, affecting to the product low yield and purity (Fickers et al., 2020). However, byproduct formation still could be reduced through a proper approach of metabolic engineering strategy and suitable adjustment of culture conditions. This major problem will subsequently advance to a challenging downstream processing, whereas a suitable yet satisfactory isolation, separation, and purification become highly crucial (Park and Ledesma-Amaro, 2023). Finally, the use of genetically modified *Y. lipolytica* in biomanufacturing faces the GMO-regulatory hurdles as other engineered organisms, makes it more difficult for food and flavour applications. In summary, the future prospects of *Y. lipolytica* in bioprocess and downstream processing appear promising in attribution to all the ongoing research, powerful foundations, and technological breakthroughs, with the ultimate goal of developing a sustainable and economically viable bioproduction process.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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