Yeasts of the Blastobotrys genus are promising platform for lipid-based fuels and oleochemicals production

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MINI-REVIEW

Yeasts of the *Blastobotrys* genus are promising platform for lipid-based fuels and oleochemicals production

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Abstract

Strains of the yeast genus *Blastobotrys* (subphylum Saccharomycotina) represent a valuable biotechnological resource for basic biochemistry research, single-cell protein, and heterologous protein production processes. Species of this genus are dimorphic, non-pathogenic, thermotolerant, and can assimilate a variety of hydrophilic and hydrophobic substrates. These can constitute a single-cell oil platform in an emerging bio-based economy as oleaginous traits have been discovered recently. However, the regulatory network of lipogenesis in these yeasts is poorly understood. To keep pace with the growing market demands for lipid-derived products, it is critical to understand the lipid biosynthesis in these unconventional yeasts to pinpoint what governs the preferential channelling of carbon flux into lipids instead of the competing pathways. This review summarizes information relevant to the regulation of lipid metabolic pathways and prospects of metabolic engineering in *Blastobotrys* yeasts for their application in food, feed, and beyond, particularly for fatty acid-based fuels and oleochemicals.

Key points

- *The production of biolipids by heterotrophic yeasts is reviewed.*
- *Summary of information concerning lipid metabolism regulation is highlighted.*
- *Special focus on the importance of diacylglycerol acyltransferases encoding genes in improving lipid production is made.*

Keywords Oleaginous yeasts · Recombinant proteins · Biofuels · *Blastobotrys* genus · Lipid feedstock · Metabolic engineering

Introduction

Budding ascomycetous yeasts (e.g., *Saccharomyces* sp., *Pichia* sp., etc.) belonging to *Saccharomycotina* subphylum are well known for their applications in basic research, food, healthcare, and agriculture. Oleaginous yeasts (such as *Yarrowia lipolytica*, *Lipomyces sp.*, *Rhodotorula sp.*) are explored for more than a decade for the promising role in replacing oleochemicals derived from fossil resources and food-grade oil plants. This is arising from their lipid production ability coupled to high-density growth on a wide range of low-cost substrates with unique physiological characteristics and availability of constantly evolving genetic tools (Juanssilfero et al. 2018; Ng 2020). *Blastobotrys adeninivorans* is another non-conventional oleaginous yeast belonging to the subphylum *Saccharomycotina*. The yeast is found in a diverse range of natural habitats. The first strain was isolated from soil and initially been named *Trichosporon adeninovorans* CBS8244^T (Middelhoven et al. 1984). Further strains have been obtained from wood hydrolysates, chopped maize silage, humus-rich soil, and fermented foods such as Pu-erh tea (Wartmann et al. 1995; Abe et al. 2008; Kunze et al. 2014). Initially described as *Trichosporon adeninivorans* by yeast taxonomic monograph (Middlehoven et al. 1984),

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and later as *Arxula adeninivorans* (Van der Walt 1990), this dimorphic yeast was finally reclassified in the genus *Blastobotrys* by Kurtzman and Robnett (2007), based on a detailed phylogenetic comparison with other related species.

Among the species described in the genus *Blastobotrys*, *B. adeninivorans* (e.g., LS3, type strain CBS 8244T and CBS 7766) has been well described (Kurtzman 2007; Kurtzmann and Robnett 2007). Recently, the LS3 strain of *B. adeninivorans* was reassigned to *B. raffinosifermentans* (Thomas et al. 2019). The LS3 strain has been exploited as a microbial biocatalyst and biosensor to detect biomarkers in medicine or pollutants in aquatic samples (Malak et al. 2016; Pham et al. 2015, Theron et al. 2014; Kasprzak et al. 2016b). It exhibits unusual metabolic and physiological characteristics, e.g., the presence of n-butanol degradation pathway; thermo-, halo-, and osmo-tolerance features; and the ability to secrete high-quality proteins including intracellular and extracellular phytases, glucoamylase, cutinases, invertase, xylosidase, cellobiases, and proteases (Bischoff et al. 2015; Wartmann and Kunze 2000; Olstorpe et al. 2009; Sano et al. 1999). Post-translational modifications like O-glycosylation and N-glycosylation are reported in this yeast (Wartmann et al. 2002). *Blastobotrys* species share nitrate assimilation and xerotolerance as common traits (Van der Walt et al. 1990). Oleaginous traits have been described for *B. adeninivorans* CBS 8244T (Sanya et al. 2020) and recently for the LS3, CBS 7377, and CBS 7766 strains of the yeast (Thomas et al. 2019). When nitrogen-limitation was imposed, lipid accumulation to above 30% was observed in *B. adeninivorans* (Thomas et al. 2019).

The LS3 strain of *B. adeninivorans* emerged as an attractive host for the expression of recombinant proteins with medical or industrial interest. An increasing number of published articles have reported the LS3 strain for producing recombinant proteins, analyzing its uric acid-reducing gene, adenine-reducing gene, guanine-reducing gene, and in improving the production of polyhydroxyalkanoates (PHAs) (polyhydroxybutyrate, PHB-V) or enantiomerically pure 1-(R)-phenyl ethanol (Jankowska et al. 2013b,2013a,2015; Litwińska et al. 2019; Trautwein-Schult et al. 2013, 2014; Rauter et al. 2015; Biernacki et al. 2017; Zhang et al. 2019). Lapeña et al. (2020) confirmed the ability of the LS3 strain to assimilate a wide range of nitrogen-containing carbon sources (e.g., proteins, purines) alongside its capacity to grow well on an enzymatically saccharified sulfite-pulped spruce wood and hydrolysates of by-products from chicken. They also reported the similarity of its amino acid profile to fishmeal and soymeal, especially for essential amino acids, hence suitable for single-cell protein production. *B. adeninivorans* CBS 8244T described earlier for its amines, purines, and adenines assimilation ability (Middelhoven et al. 1984) has now been recognized for its capacity to hydrolyze maltose, maltose-like substrates (maltulose, melezitose), and sucrose (Visnapuu et al. 2019). Kurtzman et al. (2015) highlighted the growth of both *B. adeninivorans* and *B. raffinosifermentans* on high glucose concentration (50%, 60%, and 70%) media. The dimorphic yeast described in this review alongside the CBS 8244T strain also showed promising innate potential in the bioconversion of xylose and glucose as renewable feedstock to various molecules, including lipid, acetic, and citric acid (Thomas et al. 2019; Borelli et al. 2019).

Apart from its oleagenicity and prospects in biofuel applications, the *Blastobotrys* yeasts have been studied for various other biotechnological applications, listed as follows: Katongole et al. (2017) assessed the effectiveness of solid-state fermentation with *B. raffinosifermentans* species to improve the hygienic quality and digestibility of banana peels by mono-gastric animals. *B. adeninivorans* was employed for single-cell protein (SCP) production from biogas substrate. SCP derived from microbial biomass is usually used as animal feed, particularly in aquaculture (Goldberg 1985). The yeast was suitable for SCP production and its protein content displayed essential amino acids that were similar to those of soymeal and fishmeal (Lapeña et al. 2020). The yeast has also been considered a suitable host for heterologous gene expression (Wartmann and Kunze 2000).

Microbial lipases are interesting due to their function in lipid digestion for nutrient acquisition. Stehr et al. (2003) suggested a potential benefit from these enzymes in utilizing carbohydrate-restricted environments or environments where lipids are the sole carbon source. Kumari et al. (2015) applied *B. raffinosifermentans* as a host system in shake flask cultures to express the lipase YlLip11 from *Y. lipolytica* and revealed the production of 2654 U/L lipase. The extracellular lipase Alip1p, encoded by *B. raffinosifermentans ALIP1*, is a triacylglycerol acylhydrolase that catalyzes the hydrolysis of all ester bonds in between glycerol and medium-chain fatty acids. The genetic elements related to *ALIP1* are described in Böer et al. (2005b). The *ALIP1* gene regulated by carbon source or lipophilic compounds harbored an open reading frame of 1347 bp and its expressed protein (Alip1p) containing the consensus pentapeptide motif (-Gly-X-Ser-X-Gly-) for lipid recognition exhibited a dimeric structure of 100 kDa and an optimum activity at pH 7.5 and temperature 30 °C (Böer et al. 2005b).

There are a growing number of excellent reviews on genetic engineering tools for *B. raffinosifermentans*, much like the oleaginous yeast species of the genera *Yarrowia*, *Rhodotorula*, and *Lipomyces* (Malak et al. 2016; Wang et al. 2020; Park et al. 2018; Sutanto et al. 2018). Nonetheless, owing to recent studies with new approaches and technologies, we have begun to unveil the mechanisms underlying lipid biogenesis processes on a molecular level in this yeast. Additionally, *Blastobotrys* genus has recently attracted interest as a potential source for biofuels. In the current review, we address the recent applications made with this novel non-

conventional oleaginous yeast, review genome engineering strategies, and discuss substrate constraints on lipid production and the state-of-the-art gene targeting tools available for this yeast. Most data in the literature used the name *Arxula adeninivorans*. In this review, however, we have used *Blastobotrys adeninivorans* as it remains the current taxonomically valid name.

Taxonomy and genome description

The taxonomic position of *Blastobotrys* species is summarized in Fig. 1. Generally, non-conventional yeasts under the *Saccharomycotina* subphylum include species such as *Scheffersomyces* (*Pichia*) *stipitis*, *Komagataella phaffii* (*Pichia pastoris*), *Ogataea* (Hansenula) *polymorpha*, *Kluyveromyces lactis*, *Y. lipolytica*, *Kluyveromyces marxianus*, *Ogataea thermomethanolica* (Cai et al. 2019), or *Trichosporon cutaneum* (Reiser et al. 1996). Some of them are ubiquitous to all sorts of niches, showing extreme stress tolerance phenotypes. For instance, *Trichosporon cutaneum* easily accommodates high concentrations of compounds like formic acid, acetic acid, levulinic acid, 5-hydroxymethylfurfural, and vanillin syringaldehyde, and *Pichia kudriavzevii* can sustain high furan aldehyde concentrations (Kwon et al. 2011). Phylogenetic tree reconstruction placed *B. adeninivorans* closest to the well-described non-conventional oleaginous yeast *Y. lipolytica* and coalescence-based phylogenies supported this dimorphic yeast as sister to a clade containing *Saprochaete clavata* and *Geotrichum candidum* isolate CLIB 918 (for an overview, see Shen et al. 2016). Yeast species are routinely identified using the internal transcribed spacer (ITS) region and the variable domain of the large subunit (26S) ribosomal DNA or the complete small subunit, D1/D2 domain, coupled with the gene sequences from the nearly complete large subunit (LSU) rRNA gene, mitochondrial small-subunit rRNA gene (MtSm), and cytochrome oxidase II (COXII) (Kurtzman and Robnett 2007).

B. adeninivorans LS3 is an attractive and amendable microorganism with a small genome (11.8 Mb) organized into four chromosomes—Arad1A, Arad1B, Arad1C, and Arad1D of 1659397, 2016785, 3827910, and 4300524 nucleotides, respectively. The genome size is smaller than that of *S. cerevisiae* S288c (12.1 Mb) and *Debaryomyces hansenii* CBS767 (12.2 Mb). The LS3 strain of this species is asexual with a mating-type (*MAT*) locus containing the transcription factor Matα1 (ARAD1D19294g, MTAL1), also observed in *Y. lipolytica*. However, no *Mat*α2 (MATL2) has been uncovered in contrast to *Y. lipolytica* and *S. cerevisiae*. A genome GC content of around 48.1% resembling that of *Y. lipolytica* E150 (49.0%), but significantly higher than that of *S. cerevisiae* S288c (38.3%) and *Debaryomyces hansenii* CBS767 (36.3%), has been elucidated for this strain. The complete genome sequencing has been performed by Kunze and co-workers and revealed around 6000 genes in just 11.8 Mb. A single transposable element of *Ty3/gypsy*-type long terminal repeat superfamily of retrotransposons, Taa3 (ARAD1B13860t), has been recognized within the genome alongside genes encoding for enzymes involved in β-oxidation and the glyoxylate cycle (Kunze et al. 2014).

Morphology and growth conditions

B. adeninivorans is usually cultured at 28–37 °C (Knoll et al. 2007; Kunze et al. 2014; Tsakraklides et al. 2015; Thomas et al. 2019; Sanya et al. 2020). The yeast exhibits temperature-dependent morphology characterized by the formation of budding cells below 42 °C, the appearance of pseudo-hyphae at 42 °C, and hyphae at above 42 °C, as well as the ability to grow at temperatures up to 48 °C without prior adaptation (Choudhary et al. 2016) and a few hours of survival at 55 °C (Wartmann et al. 1995). Its dimorphism is reversible with growth conditions as budding cells are re-established below 42 °C.

Cultivation mode such as shake flask (Thomas et al. 2019, Sanya et al. 2020) and fed-batch cultivation (Bernacki et al. 2017) alongside culture media encompassing selective media (Litwińska et al. 2019; Theron et al. 2014; Friedlander et al. 2016; Sanya et al. 2020) and nonselective media (Samsonova et al. 1996; Bernacki et al. 2017) are usually applied to monitor the growth of these yeasts, with the main focus on nitrogen (NH_4 Cl) and carbon sources (glucose) (Sanya et al. 2020).

Fungi (Kingdom)
Ascomycota (Phylum)
Saccharomycotina (Subphylum)
Hemoascomycetes (Class)
Endomycetales (Order)
Saccharomycetaceae (Family)
Saccharomycetoideae (Sub-family)
Blastobotrys (Genus)
Blastobotrys adeninivorans (Species)

Fig. 1 Taxonomy of *Blastobotrys adeninivorans* inspired from Gellissen et al. (2005) and Kunze et al. 2014

Oleaginous characteristics

Compared to the conventional procedures of vegetable oil production, microbial lipid production takes advantage of a short production cycle, industrial scalability of bioproducts, zero competition with food crops, independence of arable land, sustainability, and tailored processes. As such, they might circumvent the limitations posed by lipid production from oil crops and plants. For instance, Blomqvist et al. demonstrated the possibility to replace vegetable oil in the feed for Arctic char (*Salvelinus alpinus*) with microbial oils derived from *Lipomyces starkeyi* cultivated on lignocellulose hydrolysate from wheat straw (Blomqvist et al. 2018). In 2011, nearly 1500 species of ascomycete and basidiomycete yeasts belonging to 149 genera were listed and described (Kurtzman et al. 2011). Less than 30 species were characterized for their oleaginous traits (Qin et al. 2017). These oleaginous yeast species were categorized under the following classes—*Saccharomycetes*, *Cystobasidiomycetes*, *Microbotryomycetes*, *Tremellomycetes*, and *Ustilaginomycetes* (Sitepu et al. 2014).

As previously reported, lipid accumulation in oleaginous yeast occurs when there is depletion of nutrients in the culture medium, channeling the carbon flux towards cellular lipogenesis (Aggelis and Sourdis 1997). In the literature, two criteria are mainly described to characterize a yeast as an oleaginous microorganism. Firstly is the ability to store more than 20% lipid in their dry cell weight (DCW) predominantly in the form of triacylglycerol or TAG (Sitepu et al. 2014; Fakas 2017). For instance, the oleaginous red yeast *Rhodotorula glutinis* produced 70% lipid in their dry weight. Another oleaginous yeast, *Lipomyces starkeyi*, was reported to have lipid as high as 85.1% DCW (Li et al. 2013; Juanssilfero et al. 2018). Secondly is the presence of key enzymes, i.e., ATP citrate Lyse (ACL) and/or malic enzyme (MAE) responsible for the oleaginous trait (Ratledge 2014; Kamineni and Shaw 2020). The role of ACL and MAE enzymes as a supplier of acetyl-CoA and NADPH, respectively, appeared to be species-dependent for fatty acid (FA) synthesis. For instance, Dulermo et al. highlighted that the inactivation of the *ACL1* gene in *Y. lipolytica* decreases FA synthesis by 60 to 80%, confirming its essential role in FA synthesis. In contrast, inactivation of the *MAE1* gene has no effects on FA synthesis, except in a FA over-accumulating strain where it improves FA synthesis by 35% (Dulermo et al. 2015).

Although cultivation conditions for efficient *B. raffinosifermentans*-based processes (Stöckmann et al. 2009) and lipases activity were elucidated a decade ago, it is only recently that the lipid production ability and lipid-related genes have been uncovered for the yeast (Thomas et al. 2019; Sanya et al. 2020). The metabolic engineering strategies for improving its lipid production are essentially described in the works of Sanya et al. (2020). The lipid content of the unmodified wild-type strain (LS3) of *B. adeninivorans* was 30% DCW on glucose (Thomas et al. 2019). The lipid profile of *B. adeninivorans* (e.g., strains CBS 8244 and LS3), at cultivation temperatures ranging from 15 to 37 °C, contained C18:1 ($n-7$), C18:1 ($n-5$), C17:1, C18:3 ($n-3$), and C16 and C18 FAs (Olstorpe et al. 2014; Thomas et al., 2019). Interestingly, Thomas et al. demonstrated variation in FA composition of the yeast at elevated temperatures. For instance, increasing temperature from 28 to 45 °C showed an increase in C18:0 and C18:2 in the CBS 8244^T and LS3, while the relative proportions of C16:0 and C18:1 decreased (Thomas et al. 2019). In the LS3, CBS 7377, CBS 7766, and CBS 8244^T strains, the dominant FA species in lipid profile, oleic acid (18:1), ranged from 43 to 52%, depending on the strain and medium (Thomas et al. 2019). These FA species are good candidates for biodiesel quality as palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids are elucidated to be suitable FAs for biodiesel production (Knothe 2009). Of note, Thomas et al. (2019) demonstrated the capacity of this yeast to grow efficiently on both hydrophobic (oleic acid) and hydrophilic (glucose, xylose) substrates generating value-added bioproducts such as organic acids (citric acid and acetic acid). A cumulative concentration ranging from 3.4 to 10.1 g/L has been disclosed for both citric and acetic acid (Thomas et al. 2019). The anamorphic hemiascomycete yeast of this report contained fatty acid species retrieved from other oleaginous plants or yeasts and therefore can be exploited as renewable resources for industrial raw materials (Supplementary Table S1). It holds excellent promise as non-food feedstocks for the generation of bio-commodities. Some of the key findings regarding *Blastobotrys* genus are summarized in Fig. 2. In *Blastobotrys* species, total intracellular lipids were quantified by gas chromatography after lyophilizing the harvested wet cell pellets and transesterifying cellular fatty acid contents to produce fatty acids methyl esters (Thomas et al. 2019; Sanya et al. 2020). We compared the lipogenesis ability of *B. raffinosifermentans* (LS3) on some carbon substrates to that of other well-documented oleaginous yeasts in Table 1. The commonly used strains in research are summarized in Table 2.

Tools and techniques employed to engineer *Blastobotrys* yeasts

Genetic elements and vector systems developed in Blastobotrys genus

Most of the vector systems have been developed in *Blastobotrys* genus to integrate a copy of the gene of interest in its genome, for example, integration in an atrp1 (tryptophan) auxotrophic host strain of *B. raffinosifermentans*. Those elements consist of the

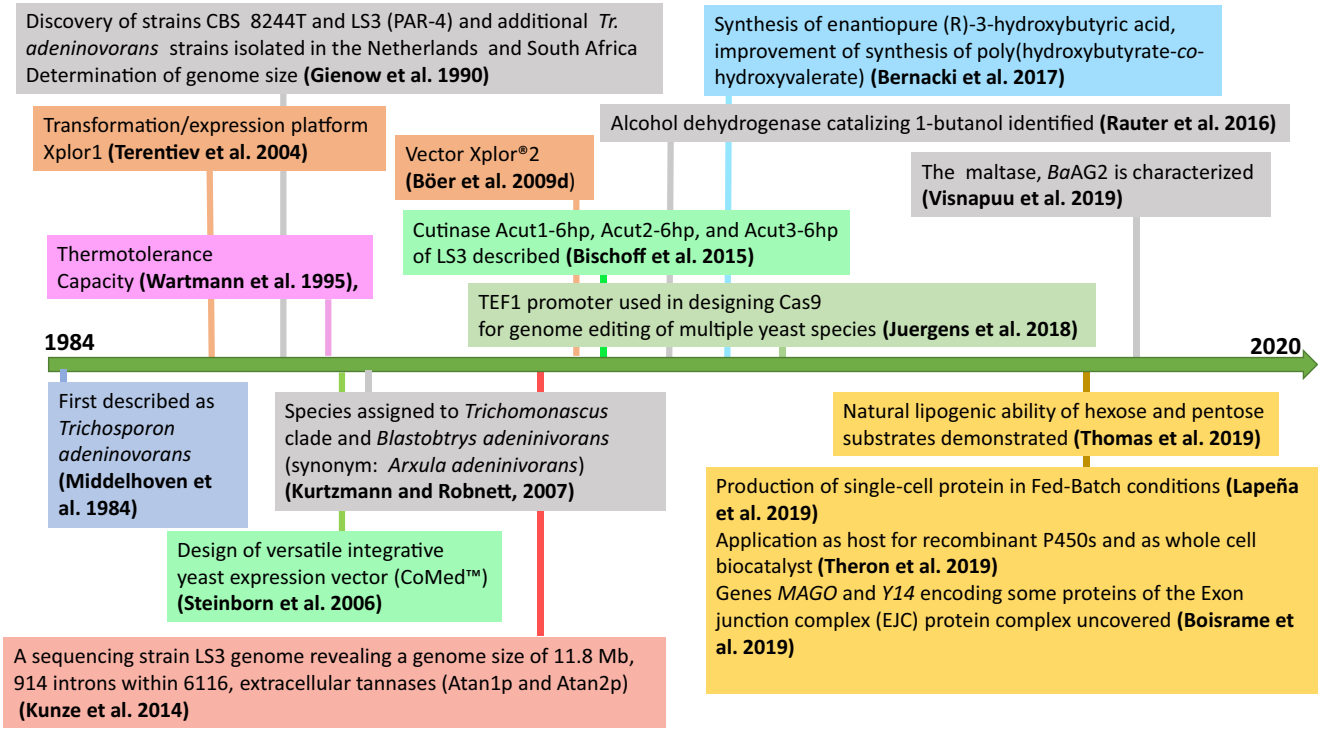

Fig. 2 Hallmarks of key events concerning *Blastobotrys* genus. Plasmids (red), other events (darkish), whole genome sequencing (brown), biofuels and industrial potential (yellow), and first species classification (blue)

ATRP1 selection marker fused to truncated ALEU2 promoter of 53 bp or TEF1 (translation-elongation factor 1α) promoter, terminator, expression vector encoding a heterologous enzyme with biotechnological interest. For instance, a high copy number (eight copies) of an amyA expression vector encoding heterologous alpha-amylase from *Bacillus amyloliquefaciens* instead of a single copy resulted in strains with superior productivity for a secreted recombinant alpha-amylase (Steinborn et al. 2007). The use of green fluorescent protein (GFP)-coding genes from the jellyfish *Aequorea victoria* as a reporter gene to assess heterologous gene expression was also reported in this yeast (Wartmann et al. 2002, Terentiev et al. 2004; Steinborn et al. 2006).

The functionality of several endogenous constitutive and inducible promoters (Supplementary Table S2), selection markers, and terminator elements (PHO5 terminator of *S. cerevisiae*, Kasprzak et al. 2016a; Steinborn et al. 2007) have been verified in *B. raffinosifermentans*. For example, the *B. raffinosifermentans AHSB4* gene promoter, encoding histone H4, was uncovered to be strongly and constitutively expressed under salt-free and salt-stress conditions (Wartman et al. 2003). On the other hand, a constitutive promoter of *TEF1* gene (encoding translation-elongation factor 1α) from *B. raffinosifermentans* that enables strong constitutive expression in various yeasts alongside genetic elements related to this gene was reported (Rösel and Kunze 1995; Steinborn et al. 2006; Terentiev et al. 2004). The Liu Y et al. (2016) demonstrated an intron in promoter's presence as relevant for improving targeted expression in *R. toruloides*. Indeed, the native *DGA1* gene of *R. toruloides* was overexpressed using two strong promoters (LDPin and GPD1) and resulted in increased lipid content by 21% in *R. toruloides* with the LDP intron promoter (LDPin) than with GPD promoter. This is also 55% higher than that reported in the *R. toruloides* wild-type strain.

The other genetic element employed in the yeast system is the transcription terminator. Eukaryotic terminators are genetically encoded elements post-transcriptionally upregulated by factors associated with *cis-elements* (e.g., GUUCG/U) within the 3′ untranslated region (UTR) (Ito et al. 2016). Both endogenous and heterologous terminators were either verified or reported functional in *B. raffinosifermentans*. For example, endogenous terminators ATRP1 have been applied to construct a selective marker module incorporated in the vector/expression system Xplor®2 or multicopy integration experiments facilitating the insertion of a yeast rDNA integrative expression cassette (YRC) or a yeast integrative expression cassette (YIC) into the genome. It is worth noting that transformants are hardly recovered when transforming *B. raffinosifermentans* by circular YRC and YIC. Simultaneously, a linearized form of YIC generated even lower transformants than a linearized form of YRC (Böer et al. 2009a, 2009d).

Like promoters and terminators, selection markers are also valuable in genome engineering. Some publications characterized the suitability of individual genes of

Table 1 Lipid production by oleaginous yeasts cultivated on various carbon sources in shake flask cultures and their comparison with the present study

Oleaginous yeast wild-type	Carbon sources	Lipid content (% dry weight)	Temperature	References
Blastobotrys adeninivorans LS3 strain vs. other oleaginous yeasts	Glucose	34.70%	28 °C, 37 °C	Thomas et al. (2019)
	Xylose	>20%		
	Arabinose	30.10%		
	Soluble starch	22.00%		
	Glycerol	16.10%		
Cryptococcus terricola	Soluble starch	26.78–36.10%	25 °C	Tanimura et al. (2014)
Lipomyces starkeyi 2512	Glucose	54.22%	25 °C	Shapaval et al. (2019)
	Xylose	52.19%		
Rhodotorula babjevae DBVPG 8058	Glucose	54.40%	25 °C	Shapaval et al. (2019)
	Xylose	23.61%		
Pseudozyma hubeiensis IPM1-10	Glucose	21.61%	28 °C	Tanimura et al. (2016)
	Xylose	24.59%		
	Arabinose	17.26%		
Prunus domestica	Glucose	15.40%	28 °C	Maina et al. (2017)
	Xylose	23.20%		
	Arabinose	14.20%		
	Galactose	21.40%		
Rhodotorula babjevae Y-SL7	Glucose	39.50%	26 °C	Guerfali et al. (2019)
	Xylose	30.09%		
	Arabinose	23.90%		
	Glycerol	29.80%		
Cutaneotrichosporon oleaginosus	Glucose	33.78%	28 °C	Awad et al. (2019)
	Xylose	13.48%		
	Arabinose	41.66%		
	Galactose	19.15%		
Lipomyces starkeyi	Glucose	79.60%	30 °C	Juanssilfero et al. (2018)
	Xylose	36.80%	28 °C	Xavier et al. (2017)
	Glycerol	35.70%	30 °C	Liu et al. (2017)
Rhodotorula toruloides	Glucose	47.51%	25 °C	Tiukova et al. (2019)
	Xylose	10.70–29.10%	28 °C	Xenopoulos et al. (2020)
	Glycerol	40.03–42.50%	30 °C	Yang et al. (2014)
Rhodotorula glutinis	Glucose	9.35–47.24%	25 °C	Maza et al. (2020)
	Xylose	12.60–33.10%	28 °C	Xenopoulos et al. (2020)
	Glycerol	10.20–12.7%	28 °C	Kot et al. (2017)
Yarrowia lipolytica	Glycerol	43.50%	30 °C	Sara et al. (2016)
	Glucose	18.00%	28 °C	Ledesma-amaro et al. (2016)

B. raffinosifermentans as auxotrophic markers. For instance, *ILV1*, from the LS3 strain, comprising 1653 bp and encoding 550 amino acids of the threonine deaminase, has been shown useful as an auxotrophic selection marker for the transformation of plasmids into this yeast (Wartmann et al. 1995, 1998). Moreover, *B. raffinosifermentans ALEU2*, encoding L-isopropylmalate dehydrogenase and harboring an open reading frame of 1086 bp, was successfully employed as an auxotrophic marker (Wartmann et al. 2003). In the experiment validating its suitability to complement the auxotrophy of a *B. raffinosifermentans* aleu2 host, the plasmid pAL-ALEU2m containing *ALEU2* as a selection marker and the 25S rDNA was used alongside the *GFP* gene, encoding an intracellular green fluorescent protein, and the HSA gene, encoding the secreted human serum albumin (Wartmann et al. 2003). The *ATRP1* encoding a phosphoribosyl anthranilate isomerase in

Table 2 *Blastobotrys adeninivorans* strains commonly used in the literature

Type/Mutant	Strain	Genotype	References
Wild-type			
Prototroph	*B. adeninivorans* LS3	Wild-type	Kunze and Kunze (1996)
	(*A. adeninivorans SBUG 724)		Wartmann and Kunze (2000)
			Lapeña et al. (2020)
	Arxula adeninivorans UOFS Y1220	Wild-type	Theron et al. (2014)
	Arxula adeninivorans SBUG724	Wild-type	Kasprzak et al. (2016a, b)
Prototroph	Arxula adeninivorans UOFS Y1220	Wild-type	Theron et al. (2014)
	A. adeninivorans CBS 8244T	wild-type	Olstorpe et al. (2014),
			Thomas et al. (2019)
	A. adeninivorans CBS 7377	Wild-type	Olstorpe et al. (2014)
			Thomas et al. (2019)
	Blastobotrys adeninivorans VKM Y-2677		Kamzolova and Morgunov (2016)
	A. adeninivorans 135		Malak et al. (2016)
	A. adeninivorans CSIR 1147	Wild-type	Wartmann et al. (1995)
	A. adeninivorans CSIR 1148	Wild-type	
	A. adeninivorans CSIR 1149	Wild-type	
	A. adeninivorans 7370	Wild-type	Thomas et al. (2019)
	A. adeninivorans 7377	Wild-type	
	Arxula adeninivorans type		Tsakraklides et al. (2015)
	strain (ATCC 76597)		
Mutants			
Leucine auxotrophic	*A. adeninivorans* G1211	[aleu2]	Böer et al. (2008), Böer et al. (2009b)
			Giersberg et al. (2012)
Auxotrophic strain	*A. adeninivorans* G1212	[aleu2 atrp1::ALEU2]	Steinborn et al. (2007), Böer et al. (2009d)
			Giersberg et al. (2012)
			Litwińska et al. (2019)
			Kasprzak et al. (2016b)
			Bischoff et al. (2015)
Arginine auxotrophic	*A. adeninivorans* G1214	[aleu2 aura3::ALEU2]	Samsonova et al. (1996)
			Böer et al. (2009b, 2009c)
	A. adeninivorans G1216	[aleu2 ALEU2::aade2]	Álvaro-Benito et al. (2013)
			Bernacki et al. (2017)
Double auxotrophic mutant	*A. adeninivorans* MS1006	[aleu2 atrp1::ALEU2 aade2::ALEU2]	Bernacki et al. (2017)
Double auxotrophic mutant	*A. adeninivorans* MS1001	[aleu2 atrp1::ALEU2 aura3::ALEU2]	Giersberg et al. (2012)
Mutant obtained by UV-light	*Arxula adeninivorans* strain G1342	[lys5-38 ilv1-2]	Kunze and Kunze (1996)
			Wartmann et al. (1998)
Mutant	*A. adeninivorans* strain, G1221 (*auox*)	[aleu2atrp1::ALEU2 auox::ATRP1]	Williams et al. (2017)
	A. adeninivorans G1224 (Δauox)	[aleu2 atrp1::ALEU2 axor::ATRP1]	Jankowska et al. (2013b)

this yeast was also used as an auxotrophic marker in experiments focused on recombinant protein production (Steinborn et al. 2006). Antibiotic resistance markers like the *Escherichia coli*-derived *hph* gene, encoding hygromycin B phosphotransferase have been used in several studies (Wartmann et al. 2003; Rösel and Kunze 1998).

Transformation methods of Blastobotrys yeast

Preparation of competent cells of *B. raffinosifermentans LS3* employed sorbitol, bicine, NaOH, and PEG-1000. Indeed, after harvesting cells cultured in rich media and washing them with water, a suspension on bicine buffer (containing 1 M sorbitol, 10 mM bicine-NaOH/pH 8.35, 3% PEG 1000, 5% DMSO), followed by centrifugation and resuspension in the same buffer generate competent cells (Kunze and Kunze 1996). After that, the linearized plasmid DNA of interest is commonly used to transform *B. raffinosifermentans LS3* competent cells.

Genome editing toolbox for Blastobotrys yeast

Among several gene-editing methods that are used currently, very few have been reported in *Blastobotrys* species, especially *B. raffinosifermentans*, on the contrary to the most thoroughly studied model yeasts, *Y. lipolytica* (Madzak 2015) and *S. cerevisiae* (Pretorius 2017; Macías et al. 2019), that have plenty of genetic engineering tools. For instance, Tsakraklides and co-workers successfully increased gene targeting in *B. raffinosifermentans* by 3–8 fold depending on

the target locus through hydroxyurea treatment (Tsakraklides et al. 2015; Galli and Schiest 1996). The development of genetic tools allowing several genetic manipulations in *B. raffinosifermentans* was mainly achieved by the Gotthard Kunze team (IPK, Gatersleben, Germany, Böer et al. 2009d). *B. raffinosifermentans* host-vector expression system was constructed for the first time by transforming auxotrophic host strain with a plasmid pAL-ATRP1-amyA containing the *ATRP1*, encoding a phosphoribosyl anthranilate isomerase isolated from the yeast *B. raffinosifermentans*, as selection marker and the 25S rDNA for targeting and equipped with an expression cassette consisting of the *Bacillus amyloliquefaciens*-derived *amyA* gene fused to the constitutive *B. raffinosifermentans*-derived TEF1 promoter and *S. cerevisiae*-derived PHO5 terminator (Steinborn et al. 2007). The sole example of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) element implicating the yeast of this report is the expression of cas9^{D147YP411T} nuclease variant under *B. raffinosifermentans*-derived TEF1 promoter (Juergens et al. 2018).

Lipid biogenesis in oleaginous yeasts

The lipogenesis mechanism in oleaginous yeasts is primarily based on the supply of acetyl-CoA and reduced nicotinamide adenine dinucleotide phosphate (NADPH), a provider of the reducing power indispensable for fatty acid synthesis. The role of the pentose phosphate pathway (PPP) has been demonstrated in lipogenesis. Glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (PGD) in the PPP provide NADPH for lipogenesis in oleaginous microbes (Chen et al. 2015a). The other known NADPH supplier for fatty acid synthesis in oleaginous microbe is the cytosolic MAE (Ratledge 2014).

Furthermore, oleaginous yeasts exhibit different mechanisms for the conversion of glucose and acetate during lipogenesis. In the case of glucose as a substrate, depleted elements from the medium such as nitrogen (Bandhu et al. 2019), phosphate (Wang et al. 2018a), or sulfur (Wu et al. 2011) usually trigger lipogenesis. Silverman et al. (2016) summarized the biosynthesis of fatty acids in oleaginous yeasts. Nutrient depletion activates the adenosine monophosphate (AMP) deaminase and NADPH supply through the oxidative pentose phosphate pathway (oxPPP) or MAE. Increased AMP deaminase activity reduces the level of mitochondrial AMP, which reduces the activity of AMP-dependent isocitrate dehydrogenase in the TCA cycle, resulting in citrate accumulation. The excess mitochondrial citrate is transported into the cytosol to generate acetyl-CoA, which is the carbon skeleton required for fatty acid biosynthesis leading to the pool of different acyl-CoAs. De novo biosynthesis of TAGs is initiated via the Kennedy pathway, where the transfer of a fatty acyl group from acyl-CoA to glycerol-sn-3-phosphate (G3P) is catalyzed by glycerol-3-phosphate acyltransferase (GPAT) to form 1-acylglycerol-sn-3-phosphate (lysophosphatidate or LPA). This step is followed by the acylation of LPA to form 1,2-diacylglycerol-sn-3-phosphate (PA), dephosphorylation of PA to form diacylglycerol (DAG). In the final step, transfer of an acyl group on DAG under diacylglycerol acyltransferase (DGAT) activity constitutes TAG. These TAGs are generally stored in lipid bodies, which are cytoplasmic lipid-enriched organelles that originate and are closely related to the endoplasmic reticulum (ER), formed by the channeling of lipids and proteins from the ER toward newly formed lipid droplets (Jackson 2019).

In the case of acetate as substrate, direct uptake through symport at higher pH (Casal et al. 1996) or passive diffusion at lower pH (Augstein et al. 2003) have been observed in yeasts. After entering the cell, dissociated acetate is converted to acetyl-CoA by the acetyl-CoA synthetase (ACS2), and the acetyl-CoA from the acetyl-CoA pool follows different pathways. It is used (1) for fatty acid synthesis through acetyl-CoA carboxylase (2) employed as a precursor for citrate formation in the mitochondrion and oxidized to carbon dioxide in the tricarboxylic acid (TCA) cycle to make energy (ATP) for the cell or (3) is converted in the glyoxylate cycle to succinate (de la Peña Mattozzi et al. 2010). Further, the ex novo pathway routinely activated in oleaginous yeasts in response to hydrophobic substrates should be noticed. It consisted of (a) the breakdown of hydrophobic compounds into free fatty acids outside the cell-mediated by lipase-catalyzed hydrolysis, (b) the transfer of free fatty acids inside the cell via transporters, and (c) the conversion of free fatty acids and their derivatives into short-chain of acyl-CoAs and acetyl-CoAs by acyl-CoA oxidases through β-oxidation (Beopoulos et al. 2009).

A communication network between organelles has been noticed during lipid biogenesis. Valm et al. (2017) indicated that lipid metabolism is dispatched between the ER for lipid synthesis, LDs for storage and transport, mitochondria and peroxisomes for β-oxidation, and lysosomes for lipid hydrolysis and recycling. Emerging evidence revealed beyond organelles' autonomous functions, a direct physical relationship between distinct organelles in eukaryotic cells. For example, the connection between LDs and peroxisomes is described by Joshi et al. (2018). They revealed that the biogenesis of LDs and peroxisome occurred at the same Pex30p subdomain. Wang and colleagues (2018b) also showed that the budding of both LDs and peroxisomes solicited the cooperation between Pex30 and the seipin complex or is inhibited otherwise. The connection between plasma membrane (PM) and ER can be seen through the yeast lipid transfer proteins (LTPs), Osh6p and Osh7p, that channel phosphatidylserine (PS) from the ER to PM via PS/phosphatidylinositol-4-phosphate (PI4P) exchange cycles. Giacomello and Pelligrini (2016) highlighted the motion of ions and lipid between mitochondria and the

ER under the mediation of MERCs (Mitochondria-ER contacts). Other contact sites identified to date between cellular organelles are summarized in Fig. 3.

Overview of lipid biogenesis genes in *Blastobotrys*

The understanding and exploration of lipid metabolism in *Blastobotrys* species are in their infancy. The lack of knowledge on the regulation of lipid accumulation in these oleaginous yeasts hinders their development as an industrial oil platform. To date, only a few reports explored the role of the significant proteins/enzymes in the lipid biosynthetic pathway of *Blastobotrys* species, unlike the case of the well-established eukaryotic model for lipid production such as *Y. lipolytica* and *Lipomyces starkeyi*. Some of the genes described for the dimorphic *B. adeninivorans* are summarized in Fig. 4 due to the critical role in their expressed membrane-bound enzymes catalyzing the last step of TAG synthesis. Thomas et al. (2019) conferred the oleaginous nature of this yeast by revealing lipid body formation along with the presence of two genes for ATP citrate lyase (ACL), suggesting their promising ability for the production of biofuel and lipids-derived compounds (Thomas 2019). Lipid bodies are essentially neutral lipid or TAG storage organelles having numerous physiological functions of the cell - including energy homeostasis, lipid metabolism, cellular toxins-trapping, and drug resistance (Chang et al. 2015). So far, the lipogenic multienzyme complex consisting of ACL (Kamineni and Shaw 2020), glycerol-3-phosphate acyltransferase (SCT1) (Tsakraklides et al. 2018), malate dehydrogenase (MDH), a key enzyme in the tricarboxylic acid (TCA) cycle (Kabran et al. 2012), Dga1p encoded by *DGA1* (ARAD1C08250g), and Dga2p encoded by *DGA2* (ARAD1D42460g) have been elucidated in LS3 strain (Sanya et al. 2020). These enzymes have been positioned on Fig. 5, which highlights the key steps in FA and TAG biosynthesis pathways. The genome of *B. adeninivorans* supposedly contains two different genes for DGAT (Thomas et al. 2019, Sanya et al. 2020). For further insights into the acyltransferases involved in lipid metabolism, we have proposed 3D structures of both Dga1p and Dga2p of *B. adeninivorans* based on local homology-based structural modeling (Supplementary Fig. 1A and 1B). Dga1p shared 13.74% sequence identity with 1-acyl-sn-glycerol-3-phosphate acyltransferase (PDB id 5kym.1) of *Thermotoga maritima*, which has been used as a template for Swiss-Model. The modeled structure highlighted the presence of α-helices in Dga1p (Supplementary Fig. 1A). For Dga2p, Swiss-Model predicted an α-helix (Supplementary Fig. 1B) sharing 33.82% sequence identity with human diacylglycerol O-acyltransferase 1 (PDB id 6vz1.1) that has been used as a template.

B. raffinosifermentans SCT1 encodes the glycerol-3-phosphate acyl-transferase (GPAT) enzyme, which is known to attach the first fatty acid onto the glycerol backbone to produce LPA in the Kennedy pathway. *SCT1* also maintains a high level of oleate in the cellular lipid composition in this yeast. When heterologously expressed in *Y. lipolytica*, it increased oleate level (Tsakraklides 2018). On the other hand, the inhibition of NAD^+-dependent isocitrate dehydrogenase linked to the increased level of α-ketoglutaric acid, an intermediate in the TCA cycle, was demonstrated in *B. adeninivorans* VKM Y-2677 cells under thiamine limitation (Komzolova and Morgunov 2016). Sanya et al. (2020) studied the diacylglycerol acyltransferases type I and type II of the membrane protein acyl-CoA: diacylglycerol acyltransferases (DGAT) in *Blastobotrys* species and identified them as essential factors in the last steps of TAG biosynthesis. Four types of diacylglycerol acyltransferases (DGATs) have been described hitherto in eukaryotes: the type 1 (DGAT1) and type 2 (DGAT2) belonging to the membrane-bound O-acyltransferase (MBOAT) family (Cases et al. 1998) and monoacylgycerol acyltransferase (MGAT) family, respectively, and the dual-specificity DGAT having both wax ester synthase (WS) and DGAT activities (Kalscheuer and Steinbuchel 2003) and a soluble cytosolic DGAT (DGAT3) (Saha et al. 2006). Of note, the Dga1p enzyme encoded by the *DGA1* gene belonging to the DGAT2 family plays a significant role in TAG biosynthesis in *Y. lipolytica* (Haïli et al. 2016). The study of the lipid metabolism pathway of *B. raffinosifermentans* (LS3 strain) highlighted the prominent role of the DGAT1 family member gene (*DGA2*). In contrast, the DGAT2 family member gene (*DGA1*) had little influence on the TAG synthesis (Sanya et al. 2020). To understand the phylogenetic relationships among the *DGA1* and *DGA2* encoded proteins expressed in *B. raffinosifermentans* and the other well-known DGAT proteins described in the literature, DGAT proteins employed by Chen et al. (2015b) alongside other DGATs retrieved from the NCBI platform (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) including those from *Rhodotorula toruloides* and *Lipomyces starkeyi* have been used to construct a phylogenetic tree. This analysis revealed that the targeted diacylglycerol acyltransferases of *B. raffinosifermentans* are clustered next to *Y. lipolytica* and away from *Lipomyces starkeyi* and *Rhotorula turoloides* (Fig. 6).

Furthermore, Stoltenburg et al. (1999) identified the gene ARFC3, encoding one subunit of the replication factor C (RF-C) complex, on chromosome 1 of the four *B. raffinosifermentans* chromosomes. *B. raffinosifermentans* appeared not to be an obligate aerobic oleaginous yeast as Sędzielewska et al. identified and characterized the gene encoding cytosolic fumarate reductase, the enzyme mediating the maintenance of redox balance through the regeneration of reduced cofactors during hypoxic growth

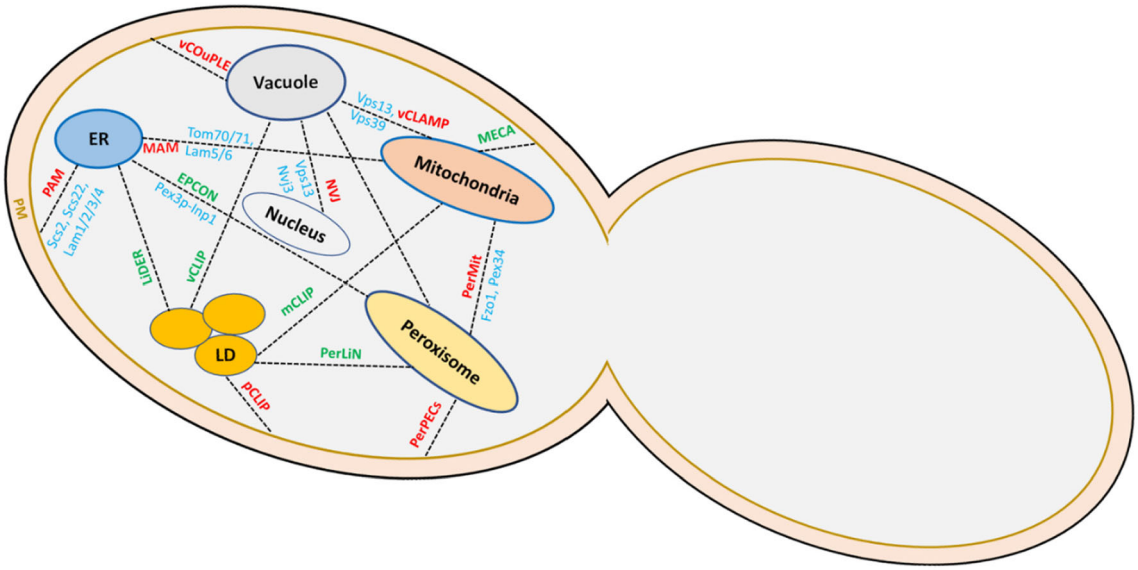

Fig. 3 A schematic diagram showing the names of identified inter-organelle contact sites. Junction names are written in green. Single protein or protein complex involved in the junction that tethers two organelles in yeast is written in blue. PM (plasma membrane), ER (endoplasmic reticulum), LD (lipid droplet). vCOuPLE (vacuole-plasma membrane contact), pCLIP (for plasma membrane contact with lipid droplets), PerPECs (for peroxisome plasma membrane contact site), PerVale (for peroxisome vacuole contact), NVJ (nuclear-vacuole junction), MAM (mitochondria-associated ER membranes), vCLAMP (vacuole and mitochondria patch), PerMit (peroxisome-mitochondria contact site), PAM (ER-PM contact), LiDER (lipid droplet-ER contact site), vCLIP (vacuole-lipid droplet contact site), mCLIP (mitochondria-lipid droplet contact site), PerLiN (peroxisome-lipid droplet contact site), and EPCON (ER-peroxisome contact site) are showed in red. The contact sites positioned on the figure are inspired by Giacomello and Pelligrini (2016), Shai et al. (2018), Joshi et al. (2018), Kakimoto et al. (2018), and Stehlik et al. (2020).

conditions, and its promoter and terminator elements (Sędzielewska et al. 2012).

Strategies for improving lipid production in oleaginous yeasts

Several strategies, including low-cost substrate utilization combined with genetic engineering and mutagenesis, have been exploited to reach a higher yield or titer of lipid production in several species of oleaginous yeasts. The ability to metabolize pentose (xylose) or/and hexose (glucose) sugars derived from lignocellulosic biomass has been widely investigated (Poontawee et al. 2017; Meesters and Eggink 1996; Yamada et al. 2017; Chattopadhyay and Maiti 2020). Other examples include *Cryptococcus terricola* with *a* lipid content of 61.96% DCW on medium with 5% starch (Tanimura et al. 2014), *Apiotrichum porosum* DSM27194 with 44.3% lipid content on volatile fatty acids from waste organics (Qian et al. 2020), and *Cystobasidium oligophagum* with 41.54%

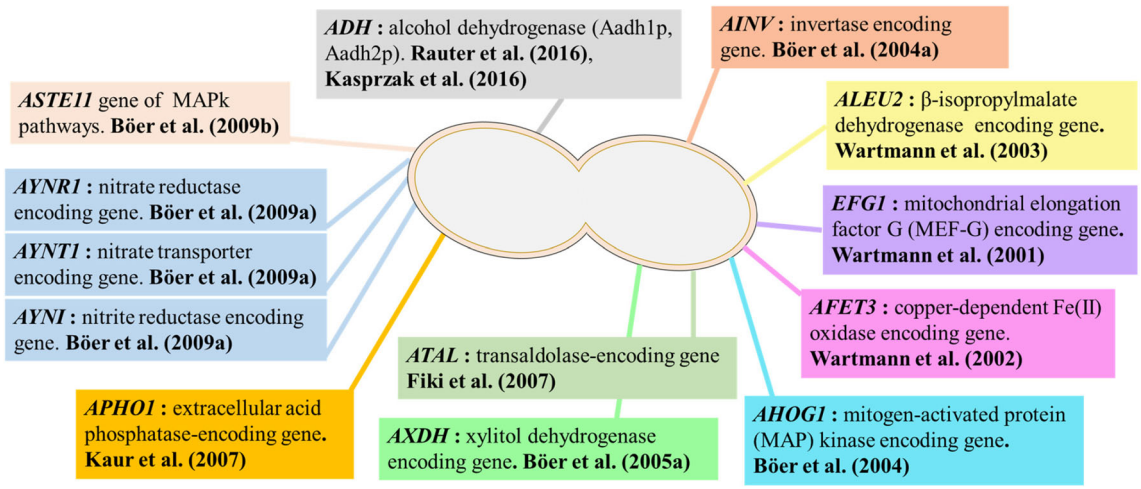

Fig. 4 Endogenous genes described for the dimorphic yeast *Blastobotrys adeninivorans*. Genes involved in the same pathway are highlighted in the same colors. The other colors are retained for the genes acting on different pathways

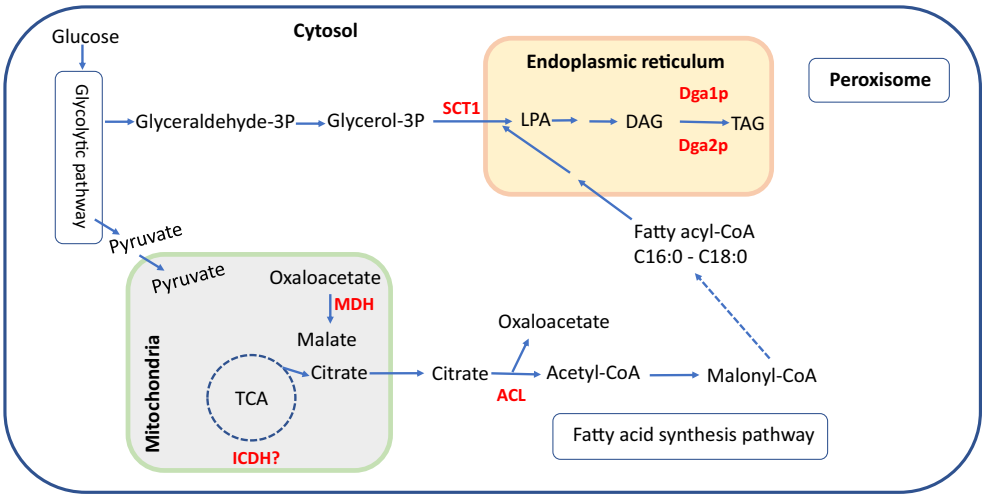

Fig. 5 Schematic diagram of lipogenesis with emphasis on acyl-CoA and TAG synthesis in the oleaginous yeast *Blastobotrys raffinosifermentans*. Abbreviations and red color are for enzymes identified so far in the literature. ACL ATP-citrate lyase, Dga1p/Dga2p diacylglycerol acyltransferase, SCT1 glycerol-3-phosphate acyltransferase, MDH malate dehydrogenase.

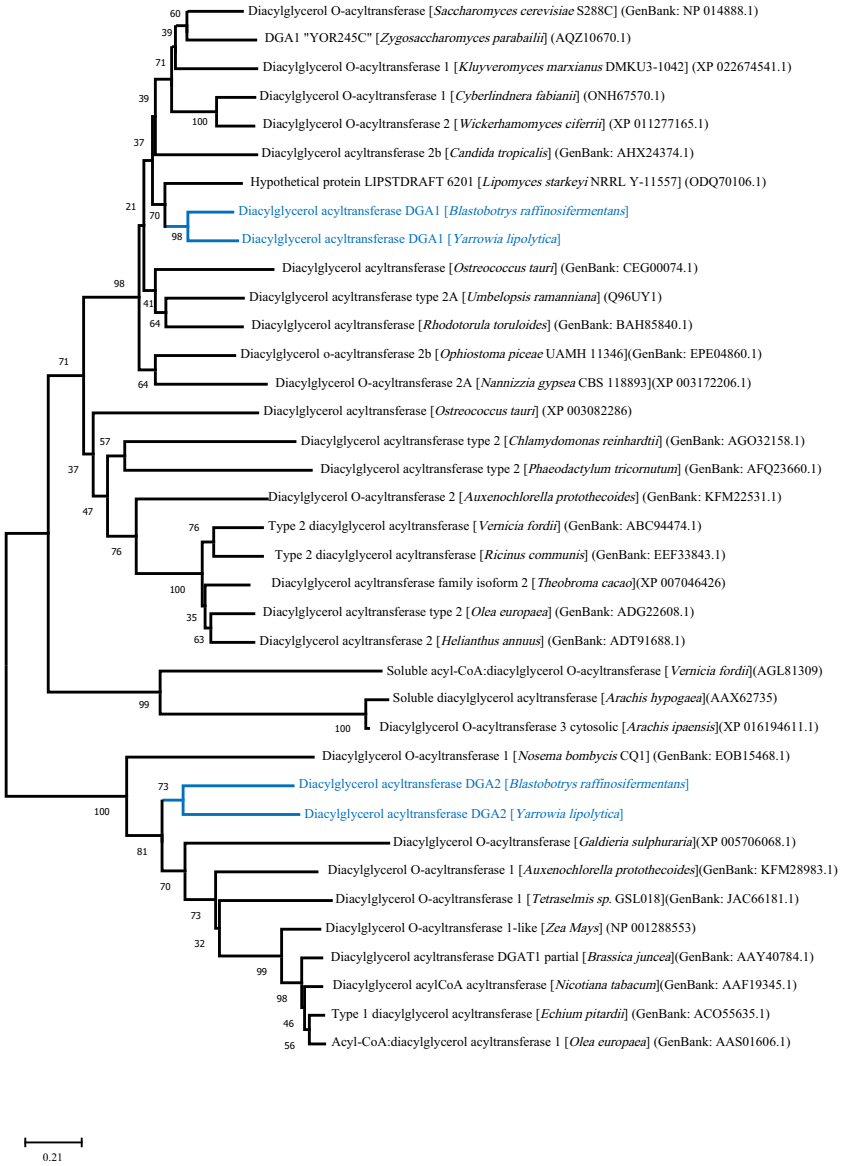

Fig. 6 Diacylglycerol acyltransferases phylogenetic relationships. Phylogenetic tree was based on 35 diacylglycerol acyltransferases from organisms described by Chen et al. (2015b) and retrieved from NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Phylogenetic trees were constructed with amino acid sequences exploiting the neighbor-joining (NJ) method employing the MEGA 7.0 software (http://www.megasoftware.net/) after a pairwise distances computation. Multiple sequence alignments were performed using the MUSCLE algorithm from MEGA 7.0. Values at the nodes represent 500 bootstrap replications (right). NCBI accession numbers except for DGA1 and DGA2 of *Y. lipolytica* and *B. raffinosifermentans* are left in the parentheses. Species are introduced in the bracket. Highlighted in blue color *Blastobotrys raffinosifermentans* diacylglycerol acyltransferases of this review

lipid on starchy materials without the use of amylolytic enzymes (Vyas and Chhabra, 2017). The natural ability of specific yeasts to grow and accumulate lipid utilizing hydrophobic substrates has been of research interest, such as in *Cryptococcus curvatus* with 69.14% DCW of lipids (Patel and Matsakas, 2019). Studies also reported specific oleaginous yeasts' capacity to simultaneously use several carbon sources (Tanimura et al. 2016; Anschau et al. 2014; Juanssilfero et al. 2018; Yamada et al. 2017), alongside the flocculation capacity (Sitepu et al. 2014). A yeast species' ability to consume sugars withstanding the inhibitory effect of chemicals is also of utmost importance as microbial behavior changes accordingly. For instance, the oleaginous yeast *L. starkeyi* reaches 68.24% of its DCW in lipids while supporting a mixture of the following growth inhibitors: 5-hydroxymethylfurfural, furfural, acetic acid, formic acid, acid levulin, vanillin, and syringaldehyde (Juanssilfero et al. 2018).

Researchers have designed constructs mostly to overexpress certain enzymes of the pathway to engineer further the strains for a higher amount of metabolite production or maneuver the carbon flux towards value-added molecule production. Overexpression is commonly carried out by integrating an additional gene copy under a strong promoter (Delic et al. 2014). Kamineni and Shaw (2020) suggested three fundamental strategies to increase TAG biosynthesis in yeast: increase the metabolic flux rate from sugar to TAG, re-design lipid pathway stoichiometry for more efficient conversion of sugar's carbon atoms and electrons into TAG, and eliminate competing by-products. Over the years, researchers have documented that the metabolic engineering for lipid production involve overexpression of enzymes of the fatty acid biosynthesis pathway/TAG biosynthesis pathway, regulation of the pathways associated with the TAG biosynthesis, TAG degradation, lipid droplet formation and lipid droplet degradation, partial blockage of competing for metabolic pathways, and the "multigene" transgenic approach to achieve these objectives (Xie et al. 2015; Liu Y et al. 2016; Ledesma-Amaro et al. 2016; Wang et al. 2020).

As previously mentioned, the DGAT enzyme plays a crucial role in TAG biosynthesis. Hence, the molecular understanding of the genes' regulatory mechanism from the DGAT family appeared to be critical for their genetic manipulation to increase lipid production in oleaginous yeasts (Haïli et al. 2016; Xue et al. 2013). Changes in gene expression from the growth phase to the lipid biosynthesis phase in oleaginous yeasts and gene expression analysis of mutants with increased lipid productivity have revealed the expression of genes important for high lipid production. DGAT overexpression has already been performed recently to get insight into the lipid metabolism of *B. adeninivorans* and *B. raffinosifermentans* species (Sanya et al. 2020). Overexpression of native DGAT1 in *B. raffinosifermentans* increased lipid production (26.5% DCW) about 1.6-fold relative to the wild-type strain (LS3, 16.9% DCW) when cultivated on glucose (Sanya et al. 2020). Overexpression or deletions of acetyl-CoA carboxylase, another key enzyme of the fatty acid biosynthetic pathway, has also been attempted (Tamano et al. 2013), along with overexpression of glycerol-3-phosphate dehydrogenase (Beopoulos et al. 2008). Knowing that palmitoleic acid and oleic acid resulted from Δ9 fatty acid desaturases activities in oleaginous yeasts and they have been disclosed in the lipid profile of the yeasts of *Blastobotrys* genus, overexpression of this desaturase can be envisioned to enhance the cellular lipid content as it was reported from *Rhodosporidium toruloides* (Tsai et al. 2019). Promising approaches to improving intracellular lipid content may also exploit the repertoire of well-established genes involved in glycolysis, β-oxidation cycle, central metabolism, and lipid metabolism that is routinely reported in oleaginous yeasts (e.g., *Yarrowia lipolytica*, *Rhodosporidium toruloides*, Beopoulos et al. 2008; Zhu et al. 2012). For example, to elucidate the relevance of the metabolic circuits associated with the enhancement of lipids in *Rhodosporidium toruloides*, Castañeda and colleagues performed the deletion of the genes expressing proteins involved in the central nitrogen metabolism (*Glutamate* dehydrogenase), the tricarboxylic acid (TCA) cycle (Isocitrate dehydrogenase), and glycolysis (Glucose-6-phosphate isomerase) (Castañeda et al. 2019). On the other hand, Yuzbasheva et al. (2017) co-expressed the gene encoding $NADP^+$-dependent glucose-6-phosphate dehydrogenase (*ZWF1*) and that encoding acyl-CoA binding protein (*ACBP*) to increase the lipid content in *Y. lipolytica*, while Wang et al. (2018c) improved lipid content in *Y. lipolytica* by inactivating *MHY1* gene encoding Mhy1p that exerts regulatory functions on lipid biosynthesis, amino acid, and nitrogen metabolism and cell cycle. The inactivation of *SCT1* as a potent way to boost fatty acyl pool can be envisioned in *Blastobotrys* genus. A similar experiment in *Y. lipolytica* contributes to the enhancement of the fatty acid ethyl esters pool identified as a promising renewable alternative for biodiesel (Ng et al. 2020). Breeding using mutagen has been used efficiently for improving lipid production in oleaginous yeasts (Liu et al. 2015, Yamada et al. 2017). For example, Takaku et al. (2021) applied ultraviolet treatment to generate *Lipomyces starkeyi* mutant capable of producing higher lipids compared to the wild-type. They also identified the increased expression of acyl-CoA synthesis- and Kennedy pathway-related genes during lipogenesis. On the other hand, Yamazaki et al. (2019) demonstrated that Percoll density gradient centrifugation is an important method recommended for the identification of high-accumulating TAG mutant strains.

Future directions and conclusion

System-level metabolic engineering that highlights the key signaling pathways regulating lipid biosynthesis is necessary to harvest the potential of *Blastobotrys* strains for developing these yeasts as an industrial platform for lipid production. Albeit the genetic parts uncovered in this review, more sophisticated genome editing methods should be designed and evaluated. Further detailed analyses of the deletion or knockout strategies to validate the role of the genes involved in circuits interfering with the lipid metabolism pathway will provide a novel insight into each gene's relative importance in the lipid biosynthesis pathway. The study of the biochemical role of lipid mediators associated with fluxomics, metabolomics, proteomics, and computational methods can help design a suitable strategy for improving the intracellular lipid content. These strategies could further pave new directions for genetic improvement tailoring fatty acid synthesis with immense food, healthcare, and biofuel industries. A co-cultivation system involving the yeast species of this genus with other oleaginous yeasts can also be envisioned.

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Author contribution DRAS has prepared manuscript, performed literature analysis, and prepared tables and figures. DO, VP, MKM, AC, and MBK have revised the manuscript. All authors read and approved the final manuscript.

Declarations

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Consent for publication Not applicable.

Conflict of interest All authors declare no competing interests.

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