The metabolism and genetic regulation of lipids in the oleaginous yeast *Yarrowia lipolytica*

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Abstract

The biotechnological potential of *Yarrowia lipolytica*, as a single cell oil-producing microorganism, is presented in this review. Although initially this yeast species was considered as a lipid-degrading, recently, it was reclassified as a lipid-producing microorganism, since it has been reported to be capable of accumulating diverse desirable fatty acids after metabolic pathway engineering. In the first part of the present document, a general revision of the oil metabolic pathways and the capacity of oil production in *Y. lipolytica* is presented. The single cell oil produced by these metabolic engineering strategies has been designed by optimization, introduction, or suppression of new pathways to increase yield on lipid production. Later on, the genetic regulation systems and the lipid composition generated by this yeast for industrial purposes are discussed. These lipids could be safely used in the chemical food and biofuel industries, due to their high proportion of oleic acid. This document emphasizes in the overviewing at *Y. lipolytica* as an ideal oil cell factory, and as an excellent model to produce single cell oil.

Keywords Lipid metabolism · Citric acid · Lipases · Hydrophobic substrates · *Y. lipolytica*

Introduction

Microbial oils have been an attractive alternative in industry to produce nutraceuticals, biofuels, cosmetic, pharmaceuticals, and other products. Currently, various microorganisms are enlisted as single cell oil producers, since they fulfill the quality standards demanded by the agro-alimentary, pharmaceutical and bioenergy industry. In most of the laboratory studies, the wide use of genetically modified model organisms is the main tool for this purpose. From the group of oil-producing organisms, the yeast *Yarrowia lipolytica* is widely used due its capability to produce large intracellular amounts of lipids which possess a high industrial and commercial value, based on their adequate proportion of unsaturated fatty acids and triglycerides. These lipids may be used as the base of biodiesel production or even as edible oil due to their high content of

unsaturated fatty acids. Besides, *Y. lipolytica* has a known genome and diverse molecular tools have been developed to exploit the metabolic engineering of this yeast in order to maximize the production of lipids.

The present review approaches general and basic genetic aspects of the synthesis, storage, and applications of the single cell lipids produced by *Y. lipolytica*. Also, in this document, this yeast stands out as a microorganism with a high metabolic production potential and with a wide market value, provided by the quality of the products that can be generated. Likewise, the main products obtained by genetic strategies directed to the synthesis and high-quality single cell oil storage, are being considered for this document.

Yarrowia lipolytica generalities and oil pathways

The generic name of *Y. lipolytica* was proposed by Van der Walt and Von Arx in 1980, in recognition of David Yarrow's work, who described this genus, and the term "lipolytica" due to its capacity to hydrolyze lipids, belonging to the family Dipodascaceae [1, 2]. Also, it is often naturally considered as a dimorphic fungus, in allusion to its two possible ways of growing: as a uniform yeast, growing favorably in an acidic

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pH; and as mycelium at a neutral pH. Some of the environmental factors, such as carbon source, nitrogen source, citrate, and the relation O_2/CO_2 , induce this morphological change [3]. Most of the strains are not able to grow at temperatures above 32 °C, considering it a non-pathogenic species [4, 5]. It is also a heterothallic organism [5, 6].

Y. lipolytica has been isolated from environmental samples rich in lipid content. Due to its capability to assimilate hydrophobic carbon sources, like n-alkanes, it can produce single cell protein and to secrete long-chain organic acids like citrate, isocitrate, and 2-oxoglutarate. These proper and phenotypic characteristics of the species *Y. lipolytica* have been utilized for the production of single-cell protein, using n-alkanes as the carbon source. Around 1960, studies about protein secretion were initiated in the laboratory of David Ogrydziak (US) and Claude Gaillardin (France), which favored the development of optimal tools to produce heterologous proteins in public and private laboratory facilities. Later, the efficiency of this yeast to utilize hydrophobic substrates encouraged Gerold Barth (Germany), Claude Gaillardin (France), and Richard Rachubinski (Canada) to employ this kind of substrates and to study the peroxisome biogenesis [5].

Also, it produces lipases, which reduce the substrate's size, allowing the growth on the area of the contact surface substrate-yeast, facilitating the lipid assimilation by the yeast [4, 5]. Alkane's assimilation starts by its hydroxylation to fatty alcohols in the endoplasmic reticulum by the cytochrome P450. Subsequently, the fatty alcohols are oxidized into fatty aldehydes by the enzyme alcohol dehydrogenase (ADH) in the endoplasmic reticulum or by the alcohol oxidase (AO) in the peroxisome. Then the aldehydes are oxidized into fatty acids by the enzyme aldehyde dehydrogenase (ALDH) in the endoplasmic reticulum or in the peroxisome (Fig. 1). The fatty

acids are activated by the acetyl-CoA synthase (ACS I) in the cytosol or by the acyl-CoA synthase II (ACS II) in the peroxisome, and are utilized for the synthesis on the membrane, in the lipids accumulation, or subsequently degraded by the β -oxidation activity in the peroxisome [7, 8]. All these characteristics make *Y. lipolytica* an ideal organism as a model organism for the study of the catabolism of the fatty acids.

On the other hand, in the last decades, it was observed that *Y. lipolytica* has the capacity to synthesize and accumulate large amounts of oils, even varying the kind of substrate to metabolize. For example, some substrates employed have been glucose, xylose, cellulose, glycerol, and others, which are described later, and have been widely studied due the high potentiality of applications on industry. This ability has been improved using genetic engineering tools that impacts on the metabolic pathways of this yeast. This combination has generated obese yeasts for lipids industry with focus to food, pharmaceutical or biofuel applications.

Yarrowia lipolytica and other oily yeasts

Some reports describe that lipids accumulated by yeasts present less unsaturation degree than those accumulated by fungi [8]. In general, oleaginous yeasts can synthesize and accumulate lipids but some species can reach up 65% w/w of their dry cell weight (DCW). Some yeast examples are *Candida*, *Lypomices*, *Rhodotorula*, *Cryptococcus*, *Trichosporon*, *Rhodospiridium*, and *Yarrowia* [9]. This cellular lipid production is associated to the type of substrate. For example, *Candida sp.* using glycerol crude, produce 50.2% w/w [10]. *Lypomices starkeyi* using as substrate cellobiose, xylose, and glucose blend can reach 52% w/w [11]; combining glucose and sewage sludge blend reach up to 68% w/w [12]; and

Fig. 1 Metabolic pathway of n-alkanes and the enzymes implicated in oxidation and hydroxylation processes in *Y. lipolytica*

glucose and xylose blend until 61.5% w/w [13]. Moreover, *Rhodotorula glutinis*, with glycerol crude, reaches 62.1% w/w [14]; using glycerol crude, reaches 53% w/w [15]. Also, *Cryptococcus curvatus* using glycerol substrate can reach 49% w/w [16] and 52.9% w/w [17] varying the culture conditions. *Cryptococcus freyschussii* using glycerol crude reaches 29.8% w/w [18]. *Trichosporon fermentans* using as substrate bagasse and hydrolysate can reach up 39.9% w/w [19]; with glucose and xylose blend, can reach up 61.7% w/w [20]; and with molasses 35.3% w/w [21]. Otherwise, *Trichosporon dermatis* using corncob hydrolysate substrate can reach 40.1% w/w [22]. Also, *Trichosporon cutaneum* with corn stover hydrolysate substrate can reach up 39.2% w/w [23]; with glucose and xylose blend, reaches 49.7% w/w and 59.1% w/w [23]. On the other hand, *Rhodospiridium toruloides*, using as substrate glucose, reaches up 67.5% w/w [24]; with glycerol crude, obtains 47.7% w/w [25], 54.3% w/w [26], and 69.5% w/w [25] varying the culture conditions; also using glucose, reaches 61.8% w/w [27] and 64.5% w/w [28]. Otherwise, *Rhodospiridium kratochvilovae*, using pulp and paper industry effluent as substrates, reaches 61.7% w/w [29]. Finally, *Y. lipolytica* has been used for lipid production using a large range of substrates at low cost. Some wild strains of *Y. lipolytica* using glycerol crude as substrate can reach up to 43% w/w [30]; with sugar cane bagasse hydrolysate can reach up 58.5% w/w [31]; utilizing decanter effluent from palm oil blend can reach up 50.8% [32]. Nevertheless, one of the main characteristics that make *Y. lipolytica* different from other yeasts is the wide metabolic genetic engineered strategies that have been developed to obtain high lipid production [33]. For example, Niehus et al. [34] engineered a strain of *Y. lipolytica* by overexpressing heterologous genes using xylose as carbon source; the oil production reached 67% w/w DCW. In other modifications using mutations in the glycerol-3-phosphate shuttle pathway, *Y. lipolytica* accumulated up to 65–75% w/w DCW [35]. Also, other pathways modifications have been targeted to obtain obese *Y. lipolytica* strains that reach lipid accumulation of up to 80% w/w DCW [33, 35–38]. These characteristics make *Y. lipolytica* an interesting and attractive yeast model for its industrial biotechnology exploitation mainly on biofuels and pharmaceutical and food applications.

Lipid metabolism in *Y. lipolytica*

The lipid accumulation and mobilization in *Y. lipolytica* depend mainly of the yeast's physiology, nutrient availability, and medium conditions, such as temperature and pH, due to its incidence in the metabolic process development:

- 1) During the exponential growth phase, lipids are synthesized and stored in membrane systems, to be later mobilized during cell growth and division. Once assimilated, the carbon from the media is distributed among the

four macromolecular groups (carbohydrate, lipids, nucleic acids, and proteins). The carbon excess in the culture medium, propitiates *Y. lipolytica* to produce vast amounts of citrate, isocitrate acid, 2-ketoglutaric acid, and pyruvic acid. Also, if the culture medium nutrients are exhausted, specifically the nitrogen source (efficient inductor of lipids accumulation), the metabolic growth functions of *Y. lipolytica* are decelerated, causing the start of an accumulative phase of lipids with citric acid as precursor [39]. However, if the carbon from the media depletes, the lipids would be mobilized for degradation; and, therefore, the lipids accumulation would always depend on the carbon source input [40].

- 2) During the stationary phase, nutrients run out, and as consequence, the non-conjugated fatty acids are slowly freed as triglycerides via peroxisome β -oxidation [31].
- 3) Likewise, the lipid production elapses if the cell is under fast conditions and suddenly it is grown in a rich medium. In this process, the free fatty acids degradation to the culture medium immediately stops [31, 41].

Another outstanding feature of the lipid-producing microorganisms is the accumulation of lipids, which is closely related with citric acid production, generated by the isocitrate dehydrogenase activity in the carboxylic acids cycle or Krebs cycle [4]. In *Y. lipolytica*, isocitrate accumulation is correlated to the concentration of citric acid, which is accumulated on in the mitochondria by the action of the aconitase enzyme. Later, it is mobilized into the cytosol by the citrate-malate pathway and then split by the ATP citrate lyase (ACL) in the Krebs cycle (codified by the ACL1 and ACL2 genes). This enzyme is responsible for the oxaloacetate production and the accumulation of the acetyl-CoA for the fatty acids synthesis [41]. These intermediaries are important during the fatty acid synthesis, considering this enzyme capable of conferring oiliness because acetyl-CoA is a key element in the lipid synthesis during the fatty acid chain elongation [42]. Furthermore, for lipid accumulation regulation, a continuous supply of malonyl-CoA (endoplasmic reticulum) or acetyl-CoA (mitochondria) is required. Malonyl-CoA could be generated by the enzyme acetyl-CoA carboxylase (ACC), being the main source of carbon atoms for the de novo synthesis of fatty acids. Acetyl-CoA carboxylase catalyzes the initial formation of malonyl-CoA starting off acetyl-CoA reductase (ACR). In every step of the fatty acid growing chain elongation, two molecules of NADPH are required, which are generated by the action of a malic enzyme (ME) through the malate to oxaloacetate catalysis in the tricarboxylic acid cycle. This enzyme is codified by the gene MAE1 (Fig. 2) [43].

NADPH synthesis and concentration in *Y. lipolytica* do not restrict lipid accumulation, rather is dependent on the supply of an abundant carbon source to maintain the lipid reserve [44]. If it is required to determine the lipids accumulation, it

Fig. 2 Metabolic pathways involved in the fatty acid synthesis during the storage and degradation of non-polar lipids like sterol esters and triglycerides or triacylglycerols [43]

is important to consider the last three enzymes (ACC, ACL, and ME), due to its role in the regulation of this process [43, 45].

Thereby, the first attempts to accumulate lipids inside the cell were performed under a β -oxidation modification, through the genes codifying for the acyl-CoA oxidase enzyme (POX1-POX6), obtaining diverse results, like the obese yeasts [5]. Nonetheless, the fatty acid synthesis process takes place at an intracellular level, where the accumulation depends on various control processes like the transcriptional level regulation, which expresses the peroxisome biogenesis genes. Also, during the fatty acid metabolism, gene manifestation is mediated by two different of transcriptional factors known as Oaf1 and Pip2. These form a heterodimer to link the response element oleate, a promoter of the fatty acids β -oxidation and the peroxisome gene. It is worth mentioning that factors Oaf1 and Pip2 activate the transcription machinery in response to the presence of oleic acid and fatty acids [46]. Thus, in the β -oxidation pathway, fatty acids are unfolded in the peroxisome through six acetyl-CoA oxidase enzymes known as Aox1-6, codified by the genes POX1-POX6; they are important due to its function, catalyzing the start and the kinetics of the total process' steps [46].

Besides, *Y. lipolytica* capacity to synthesize triglycerides requires acyl-CoA and glycerol 3-phosphate, this last one produced from glycerol or hydroxyacetone. Glycerol 3-phosphate is oxidized by a dihydroxyacetone, through the enzyme glycerol 3-phosphate dehydrogenase, generating dihydroxyacetone, an important molecule in the glucose synthesis process (gluconeogenesis). The glycerol 3-phosphate is required for triglyceride synthesis, which can be mobilized by the conversion of free fatty acids and diacylglycerol by lipase hydrolysis. The free fatty acids are degraded by the β -oxidation pathway involving the POX, MFE, and THIO genes [43].

The triglyceride catabolic activity has been found in three genes (ScTg13, ScTg14, and ScTg15) codifying for intracellular lipase production in *S. cerevisiae*. When these are localized inside the lipid droplet, the single cell oil production increases. The implementation of the Δ ScTg13 in modified *Y. lipolytica* strains lead to the specific fatty acid profiles with C14:0, C16:0, C26:0, and C18:0 in less amount. Also, two main lipases were localized in the lipid droplets, identified as Y1Tg13 and Y1Tg14. The overexpression of Y1Tg14 presented a strong predominance and inhibited the mobilization of lipids in the yeast, which can be silenced by the overexpression of Y1Tg13 due to its role as an activator of the Y1Tg14 lipase. Accordingly, *Y. lipolytica* emerges as an important producer of organic acids and enzymes (lipases), with wide possibilities for biotechnological applications in high-value commercial products [47, 48].

Key enzyme and gene lipid production in *Y. lipolytica*

Lipids biosynthesis is initially regulated by the activity of four enzymes: AMP deaminase (AMPD), ATP-citrate lyase (ACL), malic enzyme (MAE), and acetyl-CoA carboxylase (ACC), which as mentioned in the last section, promote the lipids accumulation, especially AMPD which is dependent on nitrogen concentration. When nitrogen is at low concentrations in the culture medium, isocitrate dehydrogenase stops functioning and metabolizing isocitrate; the tricarboxylic acid cycle (Krebs cycle) gets blocked and the citrate accumulation is produced in the mitochondria. This accumulation process is mediated by the mitochondrial aconitase; the citrate enters and goes out through the citrate-malate cycle and is split by ACL; this reaction in the cytosol produces a great amount of acetyl-CoA for the fatty acids synthesis. Apart from acetyl-CoA, this process requires a continuous supply of malonyl-CoA and nicotinamide adenine dinucleotide phosphate (NADP). Malonyl-CoA can be generated from the acetyl-CoA, in a reaction catalyzed by ACC (Fig. 3) [49, 50].

The metabolic engineering of *Y. lipolytica* in the fatty acid synthesis is mediated by proteins or enzymes capable of activating, accelerating, or stopping the production of lipids in the cell. For example, in the assimilation, mobilization and storage of lipids, some of the specific substrates favor the cellular development, giving place to a de novo lipid synthesis, which can be induced by nutrient shortage in the culture medium. Same is the case of nitrogen source limitation, where the cellular proliferation is inhibited and by consequence, the regulation and lipid formation induced, caused by the AOX proteins participating in the triglyceride synthesis, and later following the Kennedy pathway [51]. In this fatty acid synthesis process, the first step is the glycerol-3-phosphate (G3P) acylation by the glycerol kinase (codified by the gene GUT1), which later gets oxidized to dihydroxyacetone phosphate (DHAP) by the G3P dehydrogenase, codified by the gene

Fig. 3 Enzymes involved in the glycerol assimilation pathway with nitrogen limitation [49]

GUT2. Acetyl-CoA activation is done by the enzyme G3P acetyltransferase (codified by the gene SCT1) and the amount of lipids is regulated by the overexpression of the genes involved in the triglyceride synthesis (Table 1). During this process, high levels of G3P are required, which are induced by the transcriptional activation of the enzyme SCT1, which codifies Sn-1 acetyltransferase [50, 51].

In the next step, the free fatty acids get activated for the principal glycerol chain acylation in the triglyceride synthesis by the enzyme SCT1 to form lysophosphatidic acid (LPA), which then gets acylated by the enzyme lysophosphatidic acid acyl-transferase into phosphatidic acid (PA). Later a dephosphorylation takes place by the phosphatidic acid phosphohydrolase, the same enzyme that frees the diacylglycerol (DAG) codified by the gene DGA1, the DAG gets acylated by the diacylglycerol acyltransferase by the acyl-CoA as a donor of the acyl group or by a phospholipid and the enzyme diacylglycerol acyltransferase codified by the gene LRO1. Also, the glycerophospholipid as the donor of the acyl group gets produced and as consequence, triglycerides get accumulated [35, 50].

Furthermore, during the yeast growth, diverse metabolic processes occur involving other enzyme participation, like the acyl-CoA oxidases: six known until now (AOX1-6 codified by the genes POX1 to POX6). It is worth mentioning each AOX enzyme has a different activity and substrate specificity, being relevant due to its participation in the β -oxidation of the fatty acids because they catalyze the first steps in the peroxisome proliferation process. Previously, inside the peroxisome, a heteropentameric cofactor gets introduced; this cofactor is an AOX2P and AOX3P protein complex, which plays a key role during the peroxisome division process giving, as a result, a

relocation in the cytosol [52]. The AOX2P protein is highly efficient and it is specific for long-chain fatty acids, while AOX3P acts preferably in short-chain fatty acids. Recently, it has been shown that AOX1P and AOX6P proteins take part in the dicarboxylic acid rupture, featuring the relevance of the proteins in the metabolic regulation of those acids [50].

Lipids accumulative metabolic pathways in *Yarrowia lipolytica*

The lipid body of the cell plays a main role in the protein content circulation, which produces and degrades lipids in the surroundings of the cellular body. Because this compartment stores energy, it maintains and regulates the membrane composition [43], also it induces the initiation of lipids accumulation through essential mechanisms for the efficient adaptation and assimilation of hydrophobic substrates like n-alkanes, fatty acids, and triglycerides. During the growth in nutrient shortage, culture medium *Y. lipolytica* utilizes surfactants for the medium nutrients solubilization from the secretion of an emulsifying agent (liposan). This agent can modify the cellular surface to facilitate the adhesion of the substrate by the creation of hydrophobic droplets. Also, *Y. lipolytica* secretes lipases for the external hydrolysis of triglycerides. Later, the hydrophobic microdroplets join the cellular surface and form protuberances on it [5].

The production of the extracellular lipase has been reported since 1948 by Peters and Nelson; the synthesis is regulated by the glucose catabolic repression as a carbon source and organic nitrogen. Also, the lipase synthesis is modulated and associated to the cellular morphology; for example, in a N-acetyl

Table 1 Genes expressed in the fatty acids synthesis in *Y. lipolytica*

Gene	Genbank accession number	Enzymatic activity
<i>YGL3</i>	YALI0D17534g	Triacylglycerol lipase
<i>YGL4</i>	YALI0F10010g	Triacylglycerol lipase
<i>LIP2</i>	YALI0A20350g	Lip2p lipase
<i>LIP7</i>	YALI0D19184g	Lip7p lipase
<i>LIP8</i>	YALI0B09361g	Lip8p lipase
<i>TGL3</i>	YALI0D17534g	Triacylglycerol lipase
<i>TGL4</i>	YALI0F10010g	Triacylglycerol lipase
<i>GUT1</i>	YALI0F00484g	Glycerol kinase
<i>GUT2</i>	YALI0B13970g	G3P dehydrogenase
<i>SCT1</i>	YALI0C00209g	G3P acyl transferase
<i>DGA1</i>	YALI0E32769g	Diacylglycerol
<i>LRO1</i>	YALI0E16797g	Phospholipid: diacyl glycerol, acyl transferase
<i>POX1</i> <i>al</i> <i>POX6</i>	YALI0E32835g	Acyl-CoA oxidase

glucosamine and citrate regulator rich medium, it is observed that the presence of high levels of lipases joined to the cell, and mycelium growth is induced [53].

It is worth to mention that the main lipase (LIP2P) favors the triglycerides hydrolysis. According to Dulermo et al. [48], lipase activity was detected in the lipid body of *Y. lipolytica* and the transmembrane protein TGL4 was identified as the main intracellular lipase. The overexpression of this protein has repercussions inhibiting the mobilization of lipids; however, this can be suppressed by the expression of its interconnector and activator TGL3. Therefore, the genes of *Y. lipolytica* contributing in the fatty acid metabolism are TGL3 and TGL4, which codify for the triacylglycerol lipase [35, 43, 48].

On the other side, the existence of a protein crucial for the substrate transport to the cell has been reported; it has been found exclusively in the lipid body and could reach up to 15 to 20% of the liposome surface. This is called the PAT protein (Perilipin, Adipophilin TIP47) and allows the lipid droplets to reach the lipase nucleus by the perilipin phosphorylation, which is considered relevant in the regulation of the substrate access to the lipases [43].

Other intracellular lipases known as Lip7p and Lip8p, codified by LIP7 y LIP8 genes respectively are characterized by structuring and transforming fatty acids. Also, lipases show affinity for specific fatty acids: Lip2p with oleates (C18), Lip7p with caproates (C6), and Lip8p with caprates (C10); these enzymes are freed into the medium and are dependent on the type of substrate [43].

The effects of liposan and the lipases could be mediated through the progressive formation of numerous hydrophobic droplets found on the cellular surface of the yeast. Its function is to facilitate the substrate transport from the surface until it reaches the endoplasmic reticulum, where the alkanes are hydroxylated into alcohols by the P450 monooxygenase, localized in the membrane of the endoplasmic reticulum [54].

Composition of the fatty acids generated by *Yarrowia lipolytica*

In studies performed with *Y. lipolytica* during the lipid and fatty acid biosynthesis utilizing different substrates, concentrations up to 81.5% of neutral free fatty acids were obtained, 6.12% were monoacylglycerides, 5.32% triglycerides, and only 4.87% were diacylglycerides; after a saponification treatment, the neutral free fatty acids profile was oleic acid (C18:0) 55.55%, palmitic acid (C16:0) 17.76%, palmitoleic acid (C16:1) 14.62%, and stearic acid (C18:0) 4.39%, and only 8.27% were long-chain fatty acids (< C18:0) [40]. In general, the quantity of long-chain fatty acids like lignoceric acid (C24:0) and other products with a carbon chain longer than 24 atoms is detected and its concentration can be increased as the growth temperature rises. This causes short-chain fatty acids like capric acid (C10:0) to deplete. This way, through this parameter, the quality control of the fatty acid synthesis is possible [31].

The obtaining of long-chain fatty acids is possible through genetic engineering by the expression of the gene codifying for proteins involved in the generation of linoleic acid in *Propionibacterium acnes*, and the co-expression of the $\Delta 12$ -desaturase gene from *Mortierella alpina* in different strains of *Y. lipolytica*. The development of this modifications were performed in different strains cultured in YPD medium, obtaining different production proportions: the strain Polh-1292-*spopai-d12-7* presented a profile of palmitic acid (C16:0) 13.9% and palmitoleic acid (C16:1) 12.4%; the strain Polh-1292-*spopai-8* obtained 49.7% of oleic acid; Polh-1292-*spopai-d12-5* showed 2.6% of (C16:2) and a 62.2% of linoleic acid (C18:2); the strain Polh-1312-*spopai-d12-9* generated 1.3% of stearic acid (C18:0); and the strain Polh-1292-*spopai-d12-16* obtained a 9.8% of conjugated linoleic acid. This data is meaningful because the linoleic acid possesses diverse positive effects on human health; its production and that of other

fatty acids are achievable through the engineering presented above [55].

Current demands of the single cell oil from *Yarrowia lipolytica*

The cellular lipid production is oriented to satisfy two main necessities at a biotechnological level. In one side, derived from the current alimentary disorders and by the so-called “express” work routine, the production of ω -3 oil takes place [56]. Commercial production of ω -3 oils by *Y. lipolytica* have such importance, it has motivated researchers to modify by genetic engineering, the long-chain polyunsaturated fatty acid synthesis, such as eicosapentaenoic acid (EPA) (C20:5n-3) and docosahexaenoic acid (DHA) (C22:6n-3), due to the human health benefits it possesses. For example, it has been reported it can diminish coronary events up to 19% in patients with coronary artery diseases history [57], as well as the triglyceride concentrations in hypertriglyceridemia adult patients [58]. The modification experienced by *Y. lipolytica* in this case, was the insertion of the genes Δ -6 and Δ -9 for the stimulation of the lipid production pathways, resulting in 56.6% of EPA in the total fatty acids produced by this yeast [59].

Using this biological model, conjugated linoleic acid (CLA) production was evaluated. CLA are a group of positional dienoic geometric isomers from linoleic acid, which have been deeply studied due to the benefits to the health of those consuming them including cancer prevention, anti-atherogenic effect, anti-obesity effect, and immunologic system modulation. *Trans* 10 and *cis* 12 CLA synthesis was performed in *Y. lipolytica* through the heterologous expression of the linoleic isomerase gene from *Propionibacterium acnes*. Utilizing soybean oil (linoleic acid abundant) as a substrate, *Y. lipolytica* presented a yield of 3.1 g/L (equivalent to 16% dry weight), as well as a secretion to the culture medium of 0.9 g/L [55].

Another opportunity area for the single cell oil relies in the biofuel production, like biodiesel, despite *Y. lipolytica* has been previously exploited in the biotechnology engineering for hydrophobic substrates degradation, through a usual transformation procedure. Nowadays, it is a challenge for human society to find renewable fuel alternatives, due to the economic cost that represents the obtaining, storage and transport of the product. However, in the last years through biotechnological implementation, processes like the production of high-quality microbial oil (similar contents as elaborated biodiesel) have been developed [60].

The lipids produced by these kinds of oily species present a composition similar to vegetable oils and fats, and through a transesterification reaction employing an alkali, is possible to convert the fatty acids into alkyl ester using methanol or

ethanol as acyl acceptors, as the final step in biofuels production [44, 48, 61].

Finally, it is necessary to recognize that in response to the enormous benefits offered by this study model, studies are tending to the increase the production of unicellular lipids. The first efforts are focused on metabolic regulation, for example, the concentration and composition of the carbon source or nitrogen limitation. Studies realized by Louhasakul and Cheirsilp in 2013 [32] with *Y. lipolytica* in residual water effluents as culture medium, generated a yield of 47% dry weight of accumulated lipids in relation with C18 to C16 fatty acids compounds. The yield obtained in this case was mostly palmitic acid (C16:0, 44%), followed by oleic acid (C18:1, 39.3%) representing more than 80% of total lipids. Utilizing glycerol as a co-substrate, the oleic acid augmented to 41–44%. Therefore, implementing this kind of inductors to the media favors the fatty acids growth during the biosynthesis, being glycerol addition a variable in lipid production [32].

On the other hand, *Y. lipolytica* NCIM 3589 with a tropical marine origin and cultivated in a glucose as carbon source medium, showed similar results, with a palmitoleic acid yield of 11% and 38.6% for oleic acid. Therefore, it is possible to affirm that fatty acids synthesized in this *Y. lipolytica* strain are optimal for oil production [61].

Metabolic manipulation has given optimal results single cell oil production and strain improving has been performed through genetic engineering, for example, in PO1f, diverse metabolic regulation checkpoints were modified, like fatty acid generation and lipid production, overexpressing AMP deaminase, and ATP citrate lyase and malic enzymes. This caused an increment in acetyl-CoA generation and NADPH supply, as well as the suppression of ACCP expression, which overexpression does not significantly improve lipogenesis. Also, the enzymes DGA1p and DGA2p (acyl-CoA: diacyl glycerol acyl transferase I and II isoenzymes) were included to potentiate the catalysis of the triglycerides synthesis. The main purpose of the overexpression and suppression modifications was to reduce the fatty acid catabolism through β -oxidation depletion (*mfe1* pathway suppression) and/or peroxisome biogenesis (*pex10* suppression). Likewise, it is known that the complete restoration of the biosynthetic pathway increases the accumulation of lipids; and the overexpression of the genes *pex10*, *Mfe1*, *leucine+*, *uracil+*, and *DGA1* allows to significantly increase the microorganism lipogenesis [45].

Concluding remarks

The yeast *Y. lipolytica* is an oily organism capable of transforming, synthesizing, and accumulating lipids of high industrial value, which are recently required as essential ingredients in food and pharmaceuticals, as well as in the biofuel industry. Furthermore, its multiple metabolic capacities and

the diverse applications for the lipids products generated by this yeast, make this organism highly promising in the biotechnology industry. It is worth to mention that, due to the fatty acid composition generated by this yeast by induced metabolic processes, either by metabolic modifications, environmental conditions or genetic engineering, the biosynthetic and metabolic potential of *Y. lipolytica* is outstanding. This augurs a promising future for this yeast, making it an ideal model for the viable production of fatty acids of industrial relevance. Likewise, *Y. lipolytica* is considered a GRAS organism; the lipids generated by it can be safely employed in alimentary and pharmaceutical industries.

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Compliance with ethical standards

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