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MINI-REVIEW

Oily yeasts as oleaginous cell factories

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Abstract Oily yeasts have been described to be able to accumulate lipids up to 20% of their cellular dry weight. These yeasts represent a minor proportion of the total yeast population, and only 5% of them have been reported as able to accumulate more than 25% of lipids. The oily yeast genera include *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon*, and *Lipomyces*. More specifically, examples of oleaginous yeasts include the species: *Lipomyces starkeyi, Rhodosporidium toruloides*, *Rhodotorula glutinis*, and *Yarrowia lipolytica*. Yeast do exhibit advantages for lipid production over other microbial sources, namely, their duplication times are usually lower than 1 h, are much less affected than plants by season or climate conditions, and their cultures are more easily scaled up than those of microalgae. Additionally, some oily yeasts have been reported to accumulate oil up to 80% of their dry weight and can indeed generate different lipids from different carbon sources or from lipids present in the culture media. Thus, they can vary their lipid composition by replacing the fatty acids present in their triglycerides. Due to the diversity of microorganisms and growth conditions, oily yeasts can be useful for the production of triglycerides, surfactants, or polyunsaturated fatty acids.

Keywords Oleaginous · Yeast · *Cryptococcus* · *Rhodotorula* · *Rodosporidum* · *Candida* · Lipids · Triglycerides

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Introduction

The ability of certain microorganisms to accumulate high amounts of lipids has been known for years, but only in the last decades, real efforts have been made to unravel the underlying biochemical pathways (Beopoulos et al. 2009). Some oily yeasts (OY) described are able to accumulate lipids to levels greater than 20% of their cellular dry weight. The typical OY genera so far indentified include *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon*, and *Lipomyces*. Also, the possibility of lipid production on an industrial basis, using OY, has been previously considered (Angerbauer et al. 2008; Jacob 1992b; Li et al. 2008; Ratledge 2004; Turcotte and Kosaric 1989).

Basically, lipids are accumulated in OY as discrete fat globular deposits and can also be associated (less than 5%) with different cell organelles. Microbial lipid compounds, known as single cell oils (SCO), have industrial interest due to their particular and precise biochemical and physicochemical properties. Additionally, OY accumulate lipids as triglycerides rich in polyunsaturated fatty acids (Papanikolaou et al. 2001). These microorganisms can accumulate oil up to 80% of their dry weight, and the technology for growing OY with high oil content is well developed (Picataggio and Smittle 1979; Boulton and Ratledge 1984; Pan et al. 2009).

Yeasts can generate lipids from different carbon sources, even from lipids present in the culture media. They can, in fact, vary their lipid composition by replacing the fatty acids in the triglycerides with those present in the culture medium (Iassonova et al. 2008).

The economic feasibility of the fermentation process to produce microbial lipids is determined by the cost of the raw materials plus the fermentation processes themselves. The cost of the raw materials depends on the ratio of the

lipids produced per amount of carbon source used, whereas the cost of the fermentation process is based on the ratio of produced lipids (defined as the amount of lipids per unit of fermentation volume and per time unit) (Ykema et al. 1988). Most of these yeasts have low growth rates, so their feasibility as lipid producers relies on the maximization of the exponential phase of growth so that a high amount of biomass is produced in short fermentation periods (Jacob and Krishnamurlhyb 1990). Another factor that must be taken into account is the use of low cost carbon sources (Ykema et al. 1988). Additionally, a way of obtaining higher added value products is by generating biosurfactants, such as sophorolipids (SL) (Daniel et al. 1999; Saxena et al. 2008) and mannosylerythritol lipids (MEL) (Rau et al. 2005), or lipids with texture similar to cocoa butter (Hassan et al. 1994).

General interest

Environmental concerns, as well as the limited amount of fossil oil resources, make fossil oil use, as well as the drilling for new oil extraction, difficult to justify. Indeed, Vasudeban and Briggs (Vasudeban and Briggs 2008) predicted that at the actual rate of oil consumption, the worldwide reserve of crude oil would only last for the next 40 years.

Biodiesel, defined as the alkyl (normally methyl or ethyl) esters of long chain fatty acids derived from plant or animal fats (Li et al. 2007), may be useful as an alternative source to replace the diesel refined from fossil oil (Pan and Rhee 1986). The worldwide market for biodiesel, it is estimated as 37 billion gallons by 2016 (Li et al. 2008).

Yeasts do exhibit advantages for lipid production over other sources. Namely, their duplication times is usually lower than one hour, they are far less affected than plants by season or climate conditions, and their cultures are more easily scaled up than those of microalgae (Li et al. 2008).

Additionally, biosurfactants from OY display better properties than their chemical counterparts in terms of biodegradability and wide range of biological properties. They may be used in cosmetics, food, and bioremediation (Park et al. 1990). Hence, SLs have been studied for their surfactant, emulsifying and antimicrobial activities (Saxena et al. 2008); they also share with MEL the characteristic of being secreted into the culture medium.

Genera

Of the 600 species of yeast, only 30 have been characterized as being able to accumulate more than 25% of their dry weight as lipids (Beopoulos et al. 2009). More specifically, examples of OY species include: *Cryptococcus albidus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan*, and *Yarrowia lipolytica*, (formerly classified as *Candida lipolytica*) (Li et al. 2008) (Table 1).

Additionally, there are some other OY species feasible for oil production and thus possible biodiesel producers (Tables 1 and 2):

1. *Cryptococcus curvatus*. This yeast can grow in glycerol, reaching biomasses of 118 g/L with lipid accumulation nearing 69% in media cultures containing oils (Iassonova et al. 2008). Their typical profile of lipid accumulation is: palmitic acid (C16:0)=28 %; stearic acid (C18:0)=15%, and oleic acid (C18:1)=48% (Iassonova et al. 2008).
2. *L. starkeyi* is able to grow and accumulate lipids (ca. 73 %) on xylose, ethanol, and L-arabinose, yielding very similar amount of biomass as that obtained with glucose as the carbon source (Table 2). Their lipid accumulation profile is: C16:0=33%, C18:1=55% (Li et al. 2008).
3. *R. toruloides* is a red basidiomycete, isolated from conifers, capable of accumulating up to 76% of lipids, with a biomass production of 100 g/L (Table 2). Their lipid profile is: C16:0=24%, C18:1=55% (Li et al. 2008).
4. *R. glutinis* is able to accumulate up to 66% of its dry weight as lipids and can reach 72% lipid accumulation with glucose as the carbon source (Beopoulos et al. 2009). Biomass production may be as high as 180 g/L,

Table 1 OY taxonomy standardized for duplicity in the references used

Name	Synonym	Anamorph
Cryptococcus curvatus	*Apiotrichum curvatum Candida curvata*	
Cryptococcus terricola	*Cryptococcus terricolus*	
Rhodosporidium toruloides	*Rhodotorula gracilis*	*Rhodotorula rubescens*
Starmerella bombicola		*Candida bombicola Torulopsis bombicola*
Lipomyces tetrasporus	*Zygolipomyces lactosus*	
Yarrowia lipolytica	*Endomycopsis lipolytica Saccharomycopsis lipolytica*	*Candida lipolytica*

Table 2 Microorganisms, culture conditions, and lipid content yields reported in the references used

Name	*X* (g/L)	Percent lipid	*T* (°C)	*t* (h)	pH	C Source	N Source	Culture	Reference
Apiotrichum curvatum UfaM3	15	45.6	30	–	5.5	Glucose	MnM	CF	Hassan et al. 1993
A. curvatum Ufa25	15	40	30	150	5	Whey	NH_4Cl	F	Ykema et al. 1989
A. curvatum ATCC 20509	85	35	30	70	4.8	Lactic permeate	MnM	F	Ykema et al. 1988
A. curvatum ATCC20509	15.11	47	32	145	5.5	Lactose	MM +vitamin	*f*	Park et al. 1990
Candida 107	18.1	37.1	30	3,528	5.5	Glucose	MnM	CF	Gill et al. 1977
C. bombicola ATCC 22214	–	SL 21 g/L	26	120	6	Glucose+soy molasses+oil	Yeast Ext. +urea	F	Solaiman et al. 2004
C. bombicola	29	SL 41 g/L	30	190	7	*C. curvatus*+lactose	MM+*C. curvatus*	F	Daniel et al. 1999
C. curvata D	10.6	27	28	72	5.5	Glucose	MM	CF	Heredia and Ratledge 1988
	8.2	30	28	72	5.5	Xylose	MM	CF	
Cryptococcus curvatus	91	33.3	28	75	5.5	Glycerol	MM	Surer®	Meesters et al. 1996b
C. albidus var. aerius IBPhM y-229	–	63.4	OP	–	5	Ethanol	MnM	F	Evans et al. 1983
C. albidus var. albidus CBS 4517	26.78	46.3	20	90	5.5	Glucose	MM	CF	Hansson and Dostalek 1986
C. curvatus ATCC 20509	118	25	28	50	5.5	Glycerol	MM	F F-b	Meesters et al. 1996
C. curvatus ATTC 20509	18.4	49.7	30	24+72	5.4	Lactose+fish oil	MM	*f*	Iassonova et al. 2008
	16.1	68.9	30	24+72	5.4	Lactose+clarinol™	MM	F	
C. terricolus	16	39	25	184	5.5	Glucose	MM	F	Boulton and Ratledge 1984
Lypomyces lipofer IBPhM y-693	–	51.5	OP	–	5	Ethanol	MnM	F	Evans et al. 1983
L.starkeyi	20.5	61.5	30	120	6	Glucose+Xylose	Variable	*f*	Zhao et al. 2008
L. starkeyi DSM 70295	13.3	56.3	30	220	5	Glucose	Basal M	*f*	Angerbauer et al. 2008
	9.3	72.3	30	220	5	Glucose	Waste water	*f*	
L. starkeyi AS 2. 1390	18	30	28	96	5.8	Glucose	MM	*f*	Li et al. 2005
	20.9	20.5	28	96	5.8	Xylose	MM	*f*	
	14	24.9	28	96	5.8	L-arabinose	MM	*f*	
Pseudozyma aphidis	33	MEL 75 g/L	27	288	6.5	Soy oil	MM	F F-b	Rau et al. 2005
	30	MEL 110 g/L	27	228	6.5	Soy oil+Glucose	MM	F F-b	
Rhodosporidium toruloides	18.2	76.1	30	120	6	Glucose	MnM	*f*	Zhao et al. 2008
R. toruloides Y4	151.5	48	30	600	5.6	Glucose	MM	*f* F-b	Li et al. 2007
	106.5	67.5	30	134	5.6	Glucose	MM	F F-b	
R. toruloides AS 2. 1389	6.9	42	28	96	5.8	Glucose	MM	*f*	Li et al. 2005
	7.2	26.8	28	96	5.8	Xylose	MM	*f*	
	4.8	16.8	28	96	5.8	L-arabinose	MM	*f*	
R. toruloides ACT 10788	–	79	27	168	5	Fatty acid sodium salt	Cornsteep	*f*	Picataggio and Smittle 1979
R. toruloides Y4	127.4	61.8	30	140	5.6	Glucose	MM	F F-b	Zhao et al. 2010
Rodotorula glutinis IIP-30	22.3	66	30	120	4	Glucose	MM	F	Jacob 1992a
R. glutinis IIP-30	17.2	39	30	120	4	Molasses+glucose +sucrose	MnM	F	Johnson et al. 1995
	25.0	42	30	120	4	Molasses+glucose +sucrose	MnM	F F-b	
R. glutinis NRRL y-1091	185	40	–	–	5.5	Glucose Oxygen enriched air	MM	F F-b	Pan et al. 1986
R. glutinis AS 2. 703	5	30.2	28	96	5.8	Glucose	MM	*f*	Li et al. 2005
	6.9	12	28	96	5.8	Xylose	MM	*f*	
	4.3	4.9	28	96	5.8	L-arabinose	MM	*f*	
R. gracilis CFR-1	10	68	28	120	5	Glucose	MM	*f*	Jacob and Krishnamurlhyb 1990
R. gracilis CFR-1	13.7	59.4	–	–	5	Molasses	MnM	SF	Jacob 1991
	13.9	60.3	–	–	5	Glucose	MM	SF	
R. minuta IIP-33	15	48	30	80	4.5	Glucose	MnM	F F-b	Saxena et al. 2008

Table 2 (continued)

Name	*X* (g/L)	Percent lipid	*T* (°C)	*t* (h)	pH	C Source	N Source	Culture	Reference
Trichosporon cutaneum AS 2. 571	3.2	65.6	28	96	5.8	Glucose	MM	*f*	Li et al. 2005
	4.2	13.4	28	96	5.8	Xylose	MM	*f*	
	5.6	8.2	28	96	5.8	L-arabinose	MM	*f*	
Yarrowia lipolytica LGAM S(7)1	8.7	40	28	240	6	Industrial lipids +glycerol	MM	F	Papanikolaou and Aggelis 2002
Y. lipolytica ACA-DC 50109	15	44	28	120	6	Animal fats	MM	F	Papanikolaou et al. 2002
Zygolipomyces lactosus	–	66.5	OP	–	5	Ethanol	MnM	F	Evans et al. 1983

T Temperature, *t* time, ***X*** biomass, *MnM* mineral medium, *MM* minimal medium, *F* fermentor, *f* flask, *F-b* fed-batch, *CF* continuous fermentor, *SL* sophorolipids, *SF* solid fermentation, *MEL* mannosylerythritol lipid, *OP* optimum

when grown in rich fermentation media (Pan et al. 1986). The lipid profile is: C16:0=18%, C18:1=60 %, linolenic acid (C18:2)=12 % (Li et al. 2008).

5. *Y. lipolytica* has a profile of accumulated lipids of C16:0=11%, C18:0=28%, and C18:2=51% (Beopoulos et al. 2009). This yeast has the ability to produce saturated lipids, such as stearin (Papanikolaou et al. 2001) under certain conditions (Table 2). Their production of polyunsaturated acids (PUFA) of the C22:6 type can be attained by genetic manipulation (Beopoulos et al. 2009).

Metabolic characteristics

The biosynthetic biochemical pathways of lipid formation in OY are not very different from those present in low oil containing yeasts, such as *Saccharomyces cerevisiae*; in fact, there are no differences in glucose uptake between an OY such as *Candida* 107 and a non-oleaginous yeast (Botham and Ratledge 1979).

Rhodotorula spp. and *Cryptococcus curvatus* can accumulate between 40% and 70% of lipids, whereas *S. cerevisiae* or *Candida utilis* only accumulate 5–10% when grown under the same conditions (Meng et al. 2009). Lipid accumulation normally takes place when the OY grows in the presence of high amounts of a carbon source and the nitrogen source is kept limited. Under these conditions, the excess carbon source is channelled into lipid bodies (LB) (Fig. 1) in the form of triglycerides (TAGs). The synthesis of TAG in yeasts takes place in the endoplasmic reticulum (ER) and in the LB. In *R. glutinis* the LBs are composed of neutral lipids (88%) in the form of TAG (72%). LB's phospholipid content differs from that found in other cell areas by the fact that they are made of phosphatidylcholine (38.6%) and phosphatidylserine (43%). Likewise, their fatty acid composition is also different, since the percentage of unsaturated lipids is lower in the LB that in the rest of the cell. These results suggest that the granule membranes are in fact different from the rest of the cell membranes (Ham and Rhee 1998).

The mechanism of lipid accumulation in OY changes when glucose or lipids are used as the carbon source (Aggelis and Sourdis 1997). Non-oleaginous yeast (NOY) cultured under the same metabolic conditions tend to stop growing when the nitrogen source is exhausted; lipid synthesis occurs at low level (less than 10%) and the left over carbon source is diverted to form polysaccharides, such as starch, β-glucan, and mannan (Ratledge 2004).

When OY enters a phase of low carbon source, they generate biomass at the expense of the accumulated fat (Holdsworth et al. 1988). Under limiting nitrogen and carbon conditions, an increase in lipid synthesis causes, in turn, an increase in glucose catabolism, through the pentose phosphate pathway. On the other hand, the hydrogenase-specific activities of the pentose phosphate pathway are not affected by the variations in both the growth rate and the specific lipid production. The majority of the reducing power in these microorganisms is not generated by malate

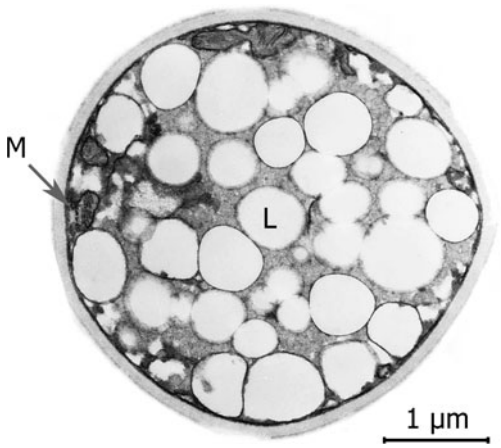

Fig. 1 *C. curvata* D grown with limiting nitrogen. Total lipid content approx. 40%. *M* mitochondrion; *L* lipid droplets (from Holdsworth et al. 1988)

enzymes, it comes mainly from the pentose pathway (Fig. 2) (Yoon et al. 1984).

One of the main differences between OY and NOY is found at the level of mitochondrial citrate, which is three to four times higher in OY than in NOY. The citrate flow, in the presence of L-malate, is higher in OY, as opposed to NOY, and OY's transporter is also more efficient (Fig. 2). Additionally, OY has a cytosolic citrate lyase not found in NOY (Evans et al. 1983); it uses ATP and Mg^{2+}, but they can, in part, be substituted by Co^{2+} and Mn^{2+} (Boulton and Ratledge 1983). One of the main factors contributing in OY lipid accumulation, as opposed to NOY, relates to their AMP N-dependent dehydrogenase. This yeasts, under limiting nitrogen conditions, experience a decrease in the amount of AMP produced, thus increasing the levels of isocitrate. This compound is then converted to citrate, by means of the mitochondrial aconitase enzyme, and the citrate transporter in the cytoplasm converts the citrate to acetyl-CoA. Hence, the ability of OY to accumulate lipids depends largely on their levels of citric acid and on their biochemical potential to metabolize it (Ratledge 2004). Indeed, the culture media nitrogen concentration is critical to induce lipid accumulation in the OYs as well as their cellular concentration of certain metabolites, such as citric acid (Beopoulos et al. 2009). The use of carbon sources such as glycerol and stearin increases their level of citric acid (Pan et al. 2009). When the concentration of this acid reaches high cellular values, it is excreted into the culture medium, lowering the pH and decreasing the amount of accumulated lipids (Papanikolaou et al. 2001). The carbon/nitrogen ratio must be controlled, particularly in continuous or fed-batch cultures, in order to direct the cellular biochemical machinery into the desirable lipid accumulation objective (Beopoulos et al. 2009).

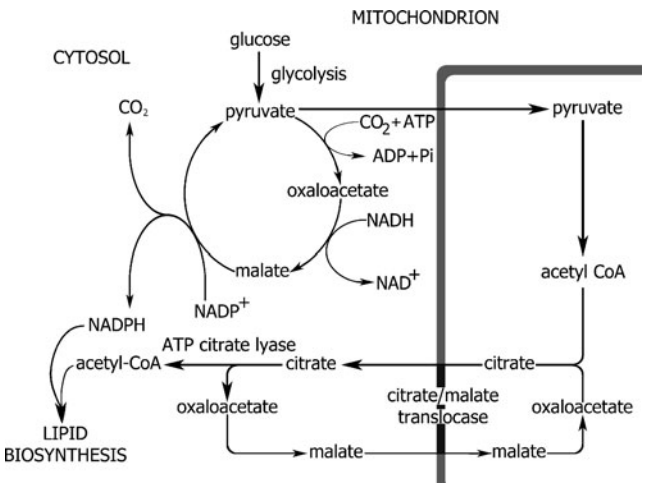

Fig. 2 Flow of citrate and malate as precursors of acetyl-CoA and NADPH for lipogenesis in oily yeast. Taken from Ratledge 2004

Synthesis of triacylglycerols in yeasts

Triglycerides (TAGs) are one of the main energy storage products in eukaryote cells. TAGs are formed by consecutive acylation of glycerol-3P by three acyltransferases, and the phosphates eliminated by means of specific phosphatases (Fig. 3). These three reactions are known as the "Kennedy pathway" for TAG biosynthesis (Angerbauer et al. 2008). Neutral lipids are stored as LB, lipid droplets or oil bodies (Fig. 1), which are covered by a phospholipid monolayer with some embedded proteins. These neutral lipids are mobilized, as required, by degradation with lipases and the products originated used for membrane formation or simply as an energy source.

In *S. cerevisiae*, both glycerol 3 phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP) can be used as substrates for the synthesis of phosphatidic acid (PA) (Fig. 3), and the enzymes required are located in LB as well as in the ER, whereas the DHAP acyltransferase is mainly located in the mitochondria. PA dephosphorylation to produce diacylglycerol (DAG) requires three phosphatidate phosphatase (PAP) isoenzymes. One of them is attached to the ER membrane, another one to the mitochondrion, and the third one is located in the cytosol. The last step of de novo synthesis of TAG in yeast can be carried out in different ways. In the reaction dependent on Acyl-CoA, the activated fatty acid binds to the DAG glycerol backbone by means of the diacylglycerol acyltransferase DgaP1 (Athenstaedt and Daum 2006); but fatty acids to be incorporated to form TAGs must be first activated by ATP. In *R. glutinis,* the reaction is catalyzed by a 35-kDa enzyme, identified as acyl–acyl carrier protein synthetase (Gangar et al. 2001).

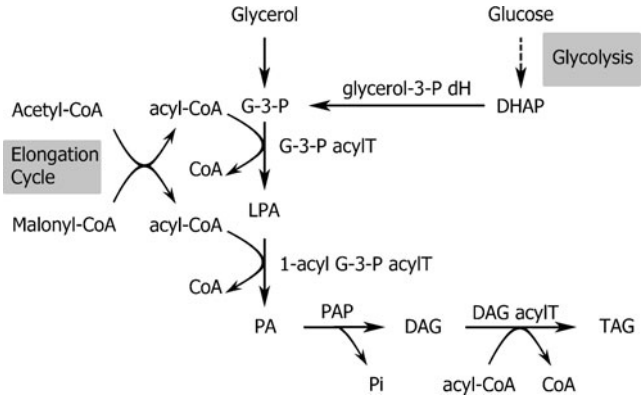

Fig. 3 Synthesis of triglycerides. *G-3-P* Glycerol-3-phosphate, *DHAP* dihydroxyacetone phosphate, *glycerol-3-P dH* glycerol-3-phosphate-dehydrogenase, *G-3-P acylT* glycerol-3-phosphate acyltransferase, *CoA* coenzyme A, *1-acyl G-3-P acylT* 1-acyl glycerol 3 phosphate acyl transferase, *LPA* lipophosphatidic acid, *PA* phosphatidic acid, *PAP* phosphatidate phosphatase, *DAG* diacylglycerol, *DAG acylT* diacylglycerol acyltransferase, *TAG* triglycerides. Modified from Ratledge 2004; Athenstaedt and Daum 2006 and Beopoulos et al. 2009

Culture conditions

The highest biomass and lipid accumulation (Table 2) occurs under certain conditions, which can be considered as critical, and these conditions include limiting amounts of nitrogen in the culture media. Although lipid accumulation is not usually associated to cell growth, the cultures (batch or fed-batch) must be strongly aerated, maintained at temperatures between 25 and 30°C, and at pH values between 3 and 6.

Lipid accumulation is influenced by the C/N ratio (optimum being close to 100), some microelements (Mg^{+2}, Zn^{+2}, Mn^{+2}, Cu^{+2}, and Ca^{+2}) (Zhao et al. 2008) and inorganic salts (Li et al. 2008; Pan and Rhee 1986). The presence of these microelements is particularly important for the activity of ATP citrate lyase in OY (Boulton and Ratledge 1983). Contrary to other OY, *Cryptococcus terricolus* accumulates lipids in the exponential phase of growth, when there is still enough amount of nitrogen in the culture medium (Boulton and Ratledge 1984). In the case of *R. glutinis*, the biomass formation can be increased by 80% using oxygen-enriched air (Nigam 1999), whereas in the case of *Apiotrychum curvatum*, the increase in lipid accumulation takes place under low oxygen conditions (Davies et al. 1990). In general, the amount of dissolved oxygen in the media is directly related to the amount of lipids accumulated (Li et al. 2008).

In *Y. lipolytica*, the accumulation of stearin during primary anabolic growth was critically influenced by the pH of the culture medium and the incubation temperature. This process was, however, independent from the nitrogen concentration in the culture medium and was increased by high carbon source concentrations and a low aeration rate (Papanikolaou et al. 2001). In *R. glutinis*, IPP30 lipid accumulation is strongly influenced by pH, with yields of 12% at pH 3, 48% at pH 5, and 44% at pH 6 (Johnson et al. 1992b).

Because most OY show low growth rates, the success of lipid accumulation relies on the rate of the exponential phase of growth, so high amount of biomass must be produced in the shortest possible time period (Jacob and Krishnamurlhyb 1990). *R. glutinis* CFR-1 is an exception to this rule and exhibits a short lag phase and a high growth rate in the first hours of growth, producing 65% lipid accumulation under these conditions (Jacob and Krishnamurlhyb 1990). In order to increase the amount of biomass, mixed fermentations in two phases are used (Heredia and Ratledge 1988; Meesters et al. 1996; Meesters et al. 1996b; Iassonova et al. 2008; Rau et al. 2005); the majority of the described processes consist of a first phase of growth with excess nitrogen source and a second phase with excess carbon source. In the case of continuous cultures, lipid accumulation is achieved by growing the oleaginous microorganisms under nitrogen-limiting conditions, at an established dilution rate. The build-up of lipid is dependent upon the correct balance between growth rate and the specific rate of lipid biosynthesis being achieved, so that the optimum amount of carbon can be diverted into lipid and the minimum possible amount into other metabolic pathways (Nigam 1999).

OY lipid production, is normally carried out in long fermentations (usually 90 h) (Angerbauer et al. 2008; Boulton and Ratledge 1984; Daniel et al. 1999; Gill et al. 1977; Hansson and Dostalek 1986; Jacob 1992a; Johnson et al. 1995; Li et al. 2007; Li et al. 2005; Pan et al. 2009; Papanikolaou et al. 2001; Papanikolaou et al. 2002; Ratledge 2004; Saxena et al. 2008; Ykema et al. 1989) (Table 2). The temperature of growth does influence the fatty acid composition present in the accumulated TAG as well as the saturation degree (Rau et al. 2005). A reduction of 5°C increases by three times the accumulation of α-linoleic in *R. glutinis* (Granger et al. 1993). The optimum pH depends on the carbon source employed for growth (Angerbauer et al. 2008).

Raw materials

It is generally accepted that lipid production from OY is industrially worthy when low-cost raw materials are used as the carbon and nitrogen sources (Pan and Rhee 1986). The ratio of accumulated biomass per used substrate (Ys) seems to be unrelated to the amount of lipid produced or the possibility of lipid accumulation (Eroshin and Krylova 1983). A variety of raw materials have been used (Table 2) including glucose (Boulton and Ratledge 1984; Gill et al. 1977; Hansson and Dostalek 1986; Hassan et al. 1993; Heredia and Ratledge 1988; Jacob 1991; Jacob 1992a; Johnson et al. 1992b; Li et al. 2007; Li et al. 2005; Pan et al. 1986; Ratledge 2004; Rau et al. 2005; Saxena et al. 2008; Zhao et al. 2008), xylose (Chistopher et al. 1983; Heredia and Ratledge 1988; Zhao et al. 2008), lactose (Chistopher et al. 1983; Daniel et al. 1999; Papanikolaou et al. 2002), L-arabinose (Li et al. 2005), mannose (Hansson and Dostalek 1986), mannitol (Hansson and Dostalek 1986), ethanol (Chistopher et al. 1983; Eroshin and Krylova 1983), dairy serum (Daniel et al. 1999; Ykema et al. 1989; Ykema et al. 1988), molasses (Jacob 1991; Johnson et al. 1995; Saxena et al. 2008), fatty acids (Johnson et al. 1992a; Lee et al. 1993; Papanikolaou et al. 2001; Picataggio and Smittle 1979; Ratledge 2004), glycerol (Meesters et al. 1996; Meesters et al. 1996b; Pan et al. 2009), solids from wheat bran fermentation (Jacob 1991), general wastewaters (Angerbauer et al. 2008), wastewaters of animal fat treatment (Papanikolaou et al. 2001), and olive oil mill wastewaters (Yousuf et al. 2010).

Glucose is the carbon source most commonly employed for the culture and lipid production by OY, although the effect of lipid consumption/accumulation cannot be estimated when such a sugar is employed (Aggelis and Sourdis 1997). High glucose concentrations inhibit the growth of some yeast, such *R. toruloides* Y4 (Li et al. 2007), and besides, the type of carbon source may influence the type of lipid accumulated. In *C. curvata* (Table 2), the proportion of fatty acids in the lipids is different in continuous cultures than in batch cultures; the proportion of fatty acids was kept constant, however, when different concentrations of nitrogen source were used. The lipid profile obtained by growing the yeast in xylose included 15% of C18:0 and 4% of C18:2, whereas when using ethanol, they contained up to 51% C18:1 and 25% of C16:0 (Evans and Ratledge 1983); but it must be pointed out that these values differ from previously reported typical lipid accumulation profiles for this yeast (Iassonova et al. 2008).

Molasses do not appear to be a good raw material for lipid production in OY, this is due to their high nitrogen content (Johnson et al. 1995). The culture medium does influence the production of lipid, as well as the lipid's properties; hence, the TAGs accumulated by OY are similar in composition to the fatty acids present in the raw materials (Lee et al. 1993). Fatty acids with carbon chains shorter than 14°C cannot be used for this purpose (Lee et al. 1992). It should be noted that when the OYs are grown in the presence of lipids as the carbon source, a rapid lipid accumulation is produced at the beginning of growth, without previous nitrogen starvation (Aggelis and Sourdis 1997). Several compounds, such as glutamate, induce the synthesis of fatty acids in *R. toruloides*, while others, such as NH_4Cl, increase carbohydrate accumulation (Evans and Ratledge 1984). These facts, together with the information that addition of citrate to the fermentation tanks noticeably increases the production of lipids in *C. albidus* (Hansson and Dostalek 1986), clearly indicate that this field requires further investigation and results must be confirmed for every independent OY.

Summary of yield data

The majority of the above-reported studies show high relative values for accumulated lipids but low biomass production. The highest lipid percentages (Table 2) were obtained with *R. toruloides* ACT 10788 (79%) in mineral medium with cornsteep and fatty acid as the carbon source (Picataggio and Smittle 1979), whereas the highest values for biomass production were obtained with *R. glutinis* NRR2 Y-1091, with values of 185 g/L and 40% lipids, and a lipid productivity of 0.88 g l^{-1} h^{-1} using oxygen-enriched air (Nigam 1999). In fed-batch fermentations containing glucose, peptone and yeast extract, *R. toruloides* was reported to accumulate up to 67.5% of lipids and a biomass of 106 g/l in only 134 h (Li et al. 2007). The same microorganism produced 79 g/l of lipids, in 140 h, with multiple fed-batch of glucose (Zhao et al. 2010). It is therefore clear that the search for new fermentable substrates able to support OY growth and resulting in high biomass production and high lipid yield is a key stone on which very well may lay the feasibility of industrial production of microbial biodiesel.

Fermentation studies of *C. curvatus* with glycerol as carbon source revealed high biomass yields but low values for lipids (Meesters et al. 1996; Meesters et al. 1996b), and the same was true for fermentations containing whey permeates (Daniel et al. 1999; Ykema et al. 1988; Nigam 1999). So far, fermentations containing sugars, such as xylose, have resulted in reduced biomass and accumulated lipids values (Table 2). Of particular relevance are the results obtained with different wastewaters, particularly those from the meat industry (Pan et al. 2009; Papanikolaou et al. 2001; Yousuf et al. 2010).

Most recently, the natural abilities of OY (mostly limited to 18:2 fatty acid production) have been enhanced by advances in genetic engineering, leading to the production of 20:4 (arachidonic acid), 20:5 (eicosapentaenoic acid), and 22:6 (docosahexaenoic acid) PUFAs in recombinant *Y. lipolytica*. These ω-3 and ω-6 fatty acids were produced by introducing and expressing heterologous genes encoding the ω-3/ω-6 biosynthetic pathway in the oleaginous host (Beopoulos et al. 2009).

Lipid extraction

In light of the information presented above, it is apparent that lipid extraction from OY is an extremely important issue. Unfortunately, it is generally accepted that there is no extraction method capable of resulting in 100% yield (Jacob 1992b).

Yeasts have several disadvantages for lipid extraction, including the presence of a thick cell wall that renders the yeast cells resistant to many solvents, as well as the possible presence of lipases in their cell extracts. In OY, most of the neutral lipids are intracellularly stored in lipid bodies. However, lipid bodies also contain other lipophylic compounds, in particular critical aromatic compounds, which are difficult to remove during lipid purification. Because of these and other difficulties, the lipid extraction methods are complex and generally responsible for the low lipid purification yields obtained (Jacob 1992b).

Most of the lipid extraction methods involve cellular breakage, either by cold or heat, or by chemical or enzymatic hydrolysis, and an extraction with organic solvents, such as petroleum ether, methanol, or chloroform.

The Soxhlet method produces one of the highest extraction yields as well as the essential CO_2 extraction (Li et al. 2001). However, due to its high cost, this method and similar ones are rarely applied at industrial scales. Oil can be extracted, at a pilot-scale, either with wet-downstream processing using ethanol:hexane and methanol:benzene, or in dry downstream processing by drying a known quantity of washed yeast cells at 70°C for 24 h and extracting oil from the dry cell pellet with ethanol: hexane (1:1) using a high-speed disperser (Nigam 1999).

Conclusions

Lipid production by OY could become a useful source of natural lipids due to the high variety of microorganisms with different profiles of lipid accumulation. Also, by manipulating the media composition or the culture conditions, TAGs enriched in a particular fatty acid could be easily produced.

Genetic manipulation of OY could, in the future, generate novel lipids that are unknown today. For example, lipids similar to cocoa butter could be produced by genetically engineered OY (Hassan et al. 1994; Ykema et al. 1989). This, in turn, could represent a very relevant factor in the future, since this type of highly appreciated commodity is diminishing worldwide due to insects and diseases in the cocoa-producing fields (Pan and Rhee 1986).

The composition of the TAG accumulated in OY is similar to that found in the raw materials. This allows for the accumulation in OY of high value lipids, such as the fatty acids present in fish oil or in conjugated oleic acid. These OY-stored fatty acids are protected from oxidation for up to 7 weeks (Iassonova et al. 2008).

In summary, due to the diversity of microorganisms and growth conditions, OYs may be useful for the production of TAG, surfactants, and/or PUFAs.

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