OCL 2013, 20(6) D603

DOI: 10.1051/ocl/2013034

OCL Oilseeds & fats Crops and Lipids

Available online at:
www.ocl-journal.org

REVIEW – DOSSIER OPEN ACCESS

MICRO-ORGANISMES PRODUCTEURS DE LIPIDES

Microorganisms as sources of oils

France Thevenieau[1] and Jean-Marc Nicaud[2,a]

[1] Direction Innovation, SOFIPROTEOL, 11-13 Rue de Monceau, CS6003, 75378 Paris Cedex 08, France
[2] INRA, UMR1319, Micalis, Domaine de Vilvert, 78352 Jouy-en-Josas, France

Received 23 July 2013 – Accepted 26 July 2013

Abstract – A number of microorganism belonging to the genera of yeast, fungi, bacteria and microalgae have ability to accumulate substantial amounts of oil, sometimes up to an even in excess of 70% of their biomass weight under specific cultivation conditions. For nearly 100 years, the commercial opportunities of using microorganisms as sources of oils have been continuously examined. Although it was evident that microbial oils could never compete commercially with the major commodity plant oils, there were commercially opportunities for the production of some of the higher valued oils. Today, with the great progress of metabolic and genetic engineering, the developments are focus on the high value oils containing important polyunsaturated or specific fatty acids. Such oils have the potential to be used in different applications area as food, feed and oleochemistry. This review is covering the related researches about different oleaginous microorganisms for lipids production and microbial oils biosynthesis process. In add, the lipid metabolism, metabolic engineering strategies to increase lipid production and the economics of microbial oils production are introduced.

Keywords: Yeast / fungus / bacteria / microalgae / microbial oils / lipids / genetic engineering

Résumé – Micro-organismes, sources de lipides. Un certain nombre de microorganismes de types levures, champignons, bactéries et microalgues présente la capacité d'accumuler des quantités significatives d'huiles jusqu'à parfois 70 % de leur poids sec dans des conditions particulières de culture. Depuis près de 100 ans, les opportunités commerciales d'utiliser ces microorganismes comme sources d'huiles ont été continuellement examinées. Bien qu'il fût évident que les huiles microbiennes ne pourraient jamais rivaliser avec les principales huiles végétales de base, des opportunités commerciales pour la production d'huiles à plus grande valeur ajoutée étaient présentes. Aujourd'hui, avec les progrès importants de l'ingénierie métabolique et génétique, les développements pré-industriels et industriels se concentrent sur les huiles à haute valeur ajoutée contenant des quantités importantes d'acides gras polyinsaturés ou fonctionnels ou des profils d'acides gras spécifiques. Ces huiles présentent un grand potentiel dans des domaines d'applications aussi divers que l'alimentation humaine, la nutrition animale ou encore l'oléochimie. Cette revue présente les caractéristiques des principaux microorganismes oléagineux d'intérêt et des huiles produites comparativement aux huiles végétales. En complément, le métabolisme lipidique et les stratégies d'ingénierie métabolique, afin d'améliorer la quantité de lipides accumulée ou le profil lipidique, ainsi que les facteurs économiques liés cette production sont discutés.

Mots clés : Levure / champignon / bactérie / microalgue / huiles microbiennes / lipides / ingénierie métabolique

Introduction

Oleaginous microorganisms are defined as oleaginous species with oil contents excess of 20% of biomass weigh. Microbial oils, also called single cell oils, are produced by some oleaginous microorganisms, such yeast, fungi, bacteria and microalgae. While the eukaryotic yeast, fungi and microalgae can synthetize triacylglycerol (TAG), which are similar with the composition of vegetable oils, prokaryotic bacteria can synthetize specific lipids.

Lipids are an effective form of energy storage with a high energy value about 39 kJ/g (9 kcal/g). Esterified as TAG and sterol esters, lipids are energy storage molecules localized in specific organelles of the cell: lipid bodies of oil seed or oleaginous microorganisms and adipose tissue of mammals. In addition to their role as energy storage, these organelles are also able to provide fatty acids and sterols for the biogenesis and maintenance of cell membranes.

Lipid metabolism is present in all microorganisms. Table 1 illustrates the wide rand of fatty acids found in oleaginous microorganisms producing TAG oils and thus can be directly

[a] Correspondence: jean-marc.nicaud@grignon.inra.fr

Table 1. Lipid accumulation and fatty acid profiles of selected oils plants and oleaginous microorganisms.

		Fatty acid compostion (% w/w)							
	Lipid content (% w/w)	**C14:0**	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**Other**
Oilseed									
Peanut	50		11	0	2	48	32		C20:0 (1%)
Rapeseed	45		4		2	62	22	10	
Sunflower	45		7		5	19	68	1	
Soybean	20		11		4	24	54	7	
Tree fruits and kernels									
Coconut	50	18	9		3	6	2		C4-C10 (15%); C12:0 (47%)
Olive	6 kg/l		13	1	3	71	10	1	C20 (1%)
Palm	50	1	44		4	38	10	1	C4-C10 (1%); C12:0 (1%)
Palm kernel		16	8		3	15	2		C4-C10 (4%); C12:0 (48%)
Microorganismes									
Yeast									
Cryptococcus albidus	60		12	1	3	73	12		
Lipomyces starkeyi	63		34	6	5	51	3		
Rhodosporidium toruloides	66		18	3	3	66			C23:0 (3%); C24:0 (6%)
Rhodotorula glutinis	72		37	1	3	47	8		
Yarrowia lipolytica	36		11	6	1	28	51		
Rhizopus arrhizus	57		18		6	22	10	12	
Fungi									
Mortierella isabellina	50		29		3	55	3	3 (n-6)	
Mucor circinelloides	25		22		5	38	10	15 (n-6)	
Pythium ultimum	48		15		2	20	16	1	C4-C10 (7%); C20:1 (4%); C20:4 (15%); C20:5 (12%)
Aspergillus terreus		2	23		trace	14	40		C21 n-3 (21%)
Pellicularia praticola			8		2	11	72		C21 n-3 (2%)
Claviceps purpurea			23		2	19	8		12-OH-C18:1 (42%)
Bacteria									
Rhodococcus opacus	19–26				3–19	6–74			
Microalgae									
Chlorella sp.	28–32		7–19	10,9	1–4	8–9	1–14	16–19	C15 (5%); C16:2 (11%)
Chlorella zofingiensis	28–32		23	2	2	36	18	8	C16:2 (7%); C16:3 (2%)
Crypthecodinium cohnii	23	13	23		3	8			C12 (3%); C22:6 (50%)
Chatoceros muelleri	31–68	18–40	5–40		0–25	0–4	0–5	0–5	C12 (6–20%); C16:2 (0–8%)
Schizochytrium linacinum	50–77	3–4	54–60		1–4				C22:5:2 (4–6%); C22:6 (29–35%)

compared, in term of their chemical compositions, to the oils obtained from oils seeds.

Compared to yeast, oleaginous fungi and microalgae have a lower lipid accumulation capacity. However, the fatty acid profiles are very different from yeast, specifically for the proportion of C20 fatty acids and omega-6 produced (Tab. 1).

The content and composition of fatty acids produced by SCO may vary depending of the type of process and the substrate used.

The main factors to evaluate the potential of a microorganism for oil production are:

1. The amount of oil produced; the more oil a microbial cell can accumulate, the more attractive it will be from a commercial viewpoint.
2. The quality of the oil produce; the profile of fatty acid varied a lot from microorganism to microorganism. Some fungi and microalgae produce polyunsaturated fatty acids with a carbon chain length and degree of unsaturation greater than those found in plants.
3. The capacity to use cheap raw material: the cost of raw material represents the overriding cost source.

These three parameters have become the key of the recent researches on the production of microbial oils.

1 Various microorganisms used

1.1 Yeast

Oleaginous yeasts, having 20% of their dry weight made up of lipids, have a fast growth and high oil content. Most oleaginous yeast can accumulate lipids at levels of more than 40% of their dry weight and as much as 70% under nutrient-limiting conditions (Tab. 1, Fig. 1). However, the lipid content differs and fatty acid profile differs between species. The best know oleaginous yeasts are typically found in genera *Candida, Cryptococcus, Lipomyces, Rhodosporidium, Rhodotorula, Rhizpus, Trichosporon* and *Yarrowia*.

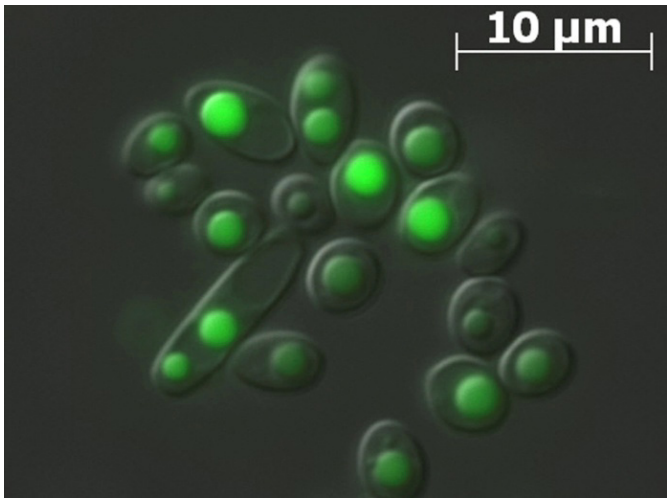

Fig. 1. Lipid accumulation in yeast. Visualization of lipid accumulation in lipid bodies during growth in glucose media by *Yarrowia lipolytica*.

Yeasts are able to utilize several different carbons sources for the production of cell mass and lipids. These sources can be glucose, xylose, glycerol, starch, cellulose hydrolysates, and industrial and municipal organic wastes. In all cases, accumulation of lipids takes place under conditions of limitations caused by a nutrient other than carbon.

When cells run out of a key nutrient, usually nitrogen, excess carbon substrate continues to be assimilated by the cells and converted into storage fat. However, the cells stop to proliferate, nitrogen is essential for the biosynthesis of proteins and nucleic acids. This pattern is observed in the lipid-accumulating yeasts and filamentous fungi, though it might not apply in photosynthetic algae, or the heterotrophic algae. In these organisms the growth rate is probably lower than the intrinsic rate of lipid biosynthesis.

The effect of a high C/N ratio on the conversion yield to lipids could be explained by the small part of the substrate used toward the basal metabolism of the cells. For example, *Rhodotorula glutinis*, present an optimal accumulation in batch culture when the C/N ratio is about 80 gsub/gNH3$^+$ (Granger, *et al.*, 1993). A higher ratio (> 80 gsub/gNH3$^+$) causes prolonged nitrogen deficiency, altering lipid synthesis and directing the metabolic flux toward the production of products such as citric acid.

Easterling (2009) explored production of lipids by the yeast *R. glutinis* on different carbon sources. The highest lipid production of 34% TAG on a dry weight basis was measured with a mixture of dextrose and glycerol as carbon source. The fraction of unsaturated fatty acids in the TAGs was dependent on carbon source, with the highest value of 53% on glycerol and lowest value of 25% on xylose. With whey permeate for production of lipids by different yeast strains, *L. starkeyi* ATCC 12659 was found to have the highest potential of accumulating lipids among *Apiotrichwn curvatum* ATCC 10567, *Cryptocooccus albidus* ATCC 56297, *L. starkeyi* ATCC 12659 and *Rhodosporidium toruloides* ATCC (Akhtar, *et al.*, 1998). The yeast *L. starkeyi* is unique in that it is know not to reutilize the lipids produced by it (Holdsworth, *et al.*, 1988).

1.2 Fungi

Many fungi species, such as *Aspergillus terreus*, *Claviceps purpurea, Tolyposporium, Mortierella alpina, Mortierella isabellina*, can also accumulate lipids. Although some types of fungi have the ability to produce lipids (Tab. 1), most fungi are explored mainly for the production of special lipids, such as docosahexaeneoic acid (DHA), gamma-linolenic acid (GLA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA).

The oleaginous fungus, *Mucor rouxii*, is known to accumulate a high level of intracellular lipids and GLA. Eroshin (2000) reported production of as much as 4.5 g l^{-1} ARA by *M. alpina* with a productivity of 19.2 mg l^{-1} h^{-1} with potassium nitrate as nitrogen source. ARA in the cells was more than 18% of dry cell mass and over 60% of the total lipids in the cells. Aki (2001) succeeded in producing 7.1 g l^{-1} ARA using the fungus *Mortierella alliacea* in a 50-L jar with a 25-L working volume; a medium containing 12% glucose and 3% yeast extract produced 46.1 g l^{-1} cells with 42.3% lipids in 7 days. The production of these polyunsaturated fatty acids in the cells is related to the age of the mycelia. Fakas (2009) found that their fraction was highest in young mycelia, and it decreased as the cells grew older. Enhanced biomass of 28.1 g l^{-1} and a lipid content of 62.4% were achieved for *T. fermentans* by Zhu (2008) with peptone as nitrogen source, glucose as carbon source, and a C/N ratio of 163.

Oleaginous fungi can also be used for the production of cocoa butter substitutes. Cocoa butter has a high saturated fatty acid content of up to 60%; of this 35% is stearic acid and 25% is palmitic acid (Dyal and Narine, 2005).

Carbon sources can strongly influence the production and composition of fatty acids in lipids of the fungi due to differences in their metabolism. Glucose, lactose, starches, oils, corn steep liquor, and agricultural produce have been used as carbon sources for production of lipids from fungi. The cellulolytic fungus, *Aspergillus oryzae* A-4, yielded a lipid content of 36.6 mg g^{-1} dry substrate by direct microbial conversion of wheat straw in suspended cultures and 62.87 mg g^{-1} dry substrate in solid substrate fermentation under optimized conditions (Hui, *et al.*, 2010).

1.3 Microalgae

Microalgae can use carbon dioxide as the carbon sources and sunlight as the energy for photoautotrophic culture, or use organic carbon as the carbon sources instead of sunlight for heterotrophic culture. And they can also use light with organic carbon as supplementary carbon sources for mixotrophic culture. Scaling up for autotrophic microalgae is more complicated, since light is needed during the cultivation process. To minimize the cost, oil production from microalgae must rely on available free sunlight, despite daily and seasonal variations in light levels

Heterotrophic microalgae are easily cultivated and controlled in normal fermenters. But, they require organic carbon sources for oil accumulation, which might limit the application of such microalgae used for high value products.

The average lipid contents of algal cells vary between 1% and 70% but can reach 90% of dry weight under certain conditions. Oil levels of 20–50% are quite common (Tab. 1).

Microalgae, such as *Chlorophyta*, *Bacillariophyceae*, have higher oil contents, and they are easier to cultivate, especially chlorella, which could be applied to industrial production, so they are the ideal energy microalgae resources. Miao (2004) acquired high lipid content of heterotrophic chlorella by heterotrophic transformation cell engineering technology, containing the lipid compounds as high as 55% of cell dry weight, which was 4 times of the autotrophic one (14%).

The growth of cells and lipid accumulation by algae under phototrophic conditions is influenced by the intensity of light, pH, dissolved oxygen concentration, fraction of carbon dioxide in sparing gas, concentration of nutrients such as nitrogen, phosphorous, silicon, and iron, and presence of organic carbon sources.

To enhance the economic feasibility of algal oil production, biomass productivity (production per unit volume per unit time), cellular lipid content, and overall lipid productivity are the three key parameters that need to be improved. These requirements are not always compatible, and in general, conditions favoring a high growth rate of cells result in a low lipid fraction in the cells and vice versa.

1.4 Bacteria

Bacteria demonstrate high cell growth rates under simple cultivation methods and some sort of bacteria can accumulate oil under some special environment. But usually, the lipid composition produced by bacteria is quite different from other microbial oils. Most bacteria just produce complex lipid, and only a few bacteria can produce. In bacteria, the most abundant class of neutral lipids are polyhydroxyalkanoic acids serving as intracellular carbon and energy storage compounds, but also few examples of substantial TAG accumulation have been reported (Tab. 1). The accumulation takes place mostly during the stationary phase of growth, *i.e.*, after the cessation of net protein synthesis. Bacterial growth in batch operations is affected by two variables: micro- and macro-nutrient limitations. Excess micronutrients support very high biomass concentrations. However, macronutrients are consumed progressively, and consequently they cause a slowing down of and finally halt in growth.

Compared to other microorganisms, many gene regulation mechanisms in fatty acid synthesis in bacteria are already understood. Therefore, it is relatively easy to use biological engineering technology, genetic engineering, and metabolic engineering to modify bacteria to improve its oil accumulation. It was reported that a metabolically engineered *Escherichia coli* could produce biodiesel directly, and the fatty acid esters concentrations of 0,7 g/l to 3,8 g/l was achieved by fed-batch fermentation using renewable carbon sources (Kalscheuer, *et al.*, 2006; Steen, *et al.*, 2010; Zhang, *et al.*, 2012). Although the yield was low, it provided a new idea for the biodiesel production.

2 Lipid metabolism and metabolic engineering

Lipid production for commercials applications depends mainly on the level of TAG accumulated, on the lipid profiles and on the capacity to use cheap raw materials. Therefore, for the improvement of a process, genetic engineering could be used. Enhance of lipid level and modification of lipid profiles were performed by targeting the four following approaches: (A) Increase the level on the two main precursors G3P and acyl-CoA; (B) boosting the TAG synthesis pathway; (C) preventing TAG remobilization and acyl-CoA degradation; (D) modification of the fatty acid profiles as described in Figure 2.

In the yeast *Yarrowia lipolytica*, when carbon is in excess and nitrogen being limiting, the carbon is redirected toward citric acid, which under the activity of the ACL, cytoplasmic ATP citrate lyase (*ACL1* and *ACL2* genes), resulted in the production of acetyl-CoA. Part of this pool is converted to malonyl-CoA by the ACC, cytoplasmic acetyl-CoA carboxylase (*ACC1* gene). Then, the multi-enzymatic complex subunits that constitute the fatty acid synthase FAS encoded by two genes (*FAS1* and *FAS2*), from malonyl-CoA add successively acetyl-CoA to elongate the acyl chain by two carbons at each cycle. The reaction is repeated until a chain length of C16 or C18 is reached releasing palmitate or stearate (C16-CoA and C18-CoA, respectively) (Fig. 2A1). Recently, Tai and Stephanopoulos (2013) increased lipid production by overexpression of the ACC. Another approach was to increase citric acid production through the inactivation of the 2-methylcitrate dehydratase encoded by the *PHD1* gene (Nicaud, *et al.*, to be published).

The second precursor, the glycerol-3 phosphate (G3P), backbone of the TAG, is part of the glycerol shuttle (Fig. 2A2). While honologs were found for the G3P dehydrogenase, the glycerol and glycerone kinases in *Y. lipolytica*, they are no honolog for the glycerol-3-phosphatase and numerous honologs for the glycerol dehydrogenase (Dulermo and Nicaud, 2011). This highlight differences for the glycerol shuttle compared to *Saccharomyces cerevisiae* which certainly participate in the oleagenoucity of *Yarrowia*. Indeed, we have previously shown that *Y. lipolytica* presents an original glycerol metabolism, dedicated to the synthesis of G3P – not glycerol with G3P having an important role into lipid accumulation. Indeed, both, deletion of the glycerol-3-Phosphatase *GUT2* (Beopoulos, *et al.*, 2008) and overexpression of *GPD1* resulted in a high improvement of TAG accumulation (Dulermo and Nicaud, 2011).

The second limiting step in the Kennedy pathway is at the last step of TAG synthesis (Fig. 2B). In yeasts, they are a diacylglycerol acyl transferase Dga1p with acyl-CoA as an donor, which belong to the DGAT2 family. In contrast, in the yeast *Y. lipolytica*, they are a second diacylglycerol acyl transferase Dga2p, which belong to the DGAT1 family. Overexpression in *Y. lipolytica* of either DGA1 or DGA2 increases TAG synthesis without modification of the fatty acid profile (Beopoulos, *et al.*, 2011). Combining overproduction of acyl-CoA by overexpression of ACC and increasing TAG synthesis by overexpression of DGA2 have been recently reported by Tai and Stephanopoulos (2013).

The third approach to improve TAG accumulation is to abolish free fatty acid (FFA) and Acyl-CoA degradation through inactivation of the beta oxidation (Fig. 2C1) or the inactivation of TAG degradation (Fig. 2C2). *Y. lipolytica* is the yeast having the highest content of acyl CoA encoding gene (six genes *POX1* to *POX6*; Wang, *et al.*, 1999). Deleting of

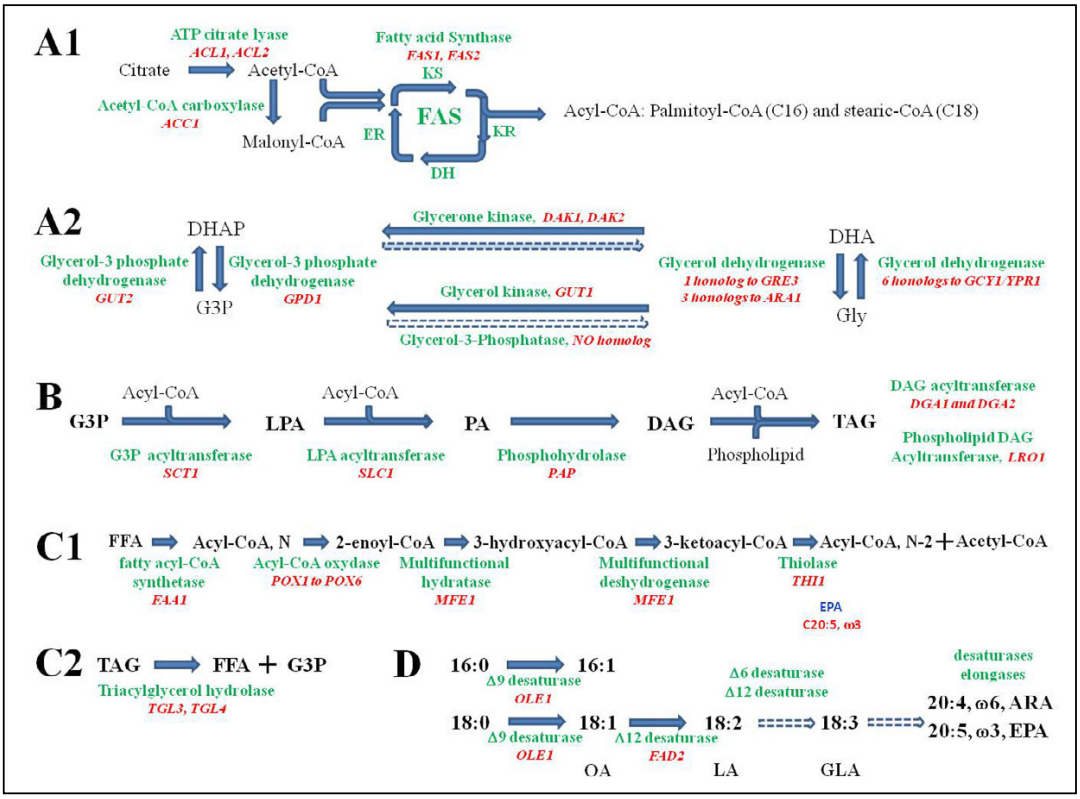

Fig. 2. Pathways targeted by metabolic engineering for triglyceride production in yeast. (A) The synthesis of the acyl-CoA precursor (A1) start with the production of citrate from the TCA cycle, secreted from the mitochondria, which is then converted to acetyl-CoA by the ATP citrate lyase (*ACL1* and *ACL2*). Part of the acetyl-CoA is converted to malonyl-CoA by the acetyl-CoA carboxylase (*ACC1*). Synthesis of fatty acids (Acyl-CoA, mainly palmitoyl-CoA and stearic-CoA) is catalyzed, through elongation cycles, by the fatty acid synthase (*FAS1* and *FAS2*) consisting of four successive reactions by the ketoacyl synthase (KS domain), further modified at the β-carbon position by ketoacyl reductase (KR), dehydratase (DH) and the enoyl reductase (ER) from the basic blocks acetyl-CoA and malonyl-CoA. (A2) The synthesis of the glycerol-3-phosphate (G3P) been part of the "gycerol pathway" that regulate the concentration of the glycerol (Gly), glycerol-3-Phosphate (G3P), dihydroxyacetone phosphate (DHAP) and dihydroxyacetone (DHA). Dihydroxyacetone phosphate (DHAP) is converted to glycerol-3-phosphate (G3P), by the NAD^+-dependent G3P dehydrogenases (GPD1) and the reverse reaction G3P, in the mitochondria, is converted to DHAP by the mitochondrial FAD^+-dependent G3P dehydrogenase (GUT2). Catabolism of glycerol depends on two pathways: (i) the "DHA pathway" in which glycerol is converted in dihydroxyacetone (DHA) by glycerol dehydrogenases (GCY1/YPR1, ARA1 and GRE3). DHA is then phosphorylated to DHAP by the glycerone kinases (DAK1 and DAK2) and (ii) the "G3P pathway" in which glycerol can be phosphorylated into G3P, by the glycerol kinase (GUT1). In *Y. lipolytica*, they are an expansion of honolog genes coding for glycerol dehydrogenase and the absence of homolog to the glycerol-3 phosphatase (GPP1 and GPP2 in *Sacharomyces cerevisiae*). (B) To boost the TAG synthesis pathway. In *Y. lipolytica*, triacylglycerol (TAG) synthesis follows the Kennedy pathway. FFA are activated to coenzyme A (acyl-CoA) and used for the acylation of the glycerol-3-P backbone to synthesize TAG. In the first step of TAG assembly, G3P is acylated by G3P acyltransferase (SCT1) to lysophosphatidic acid (LPA), which is then further acylated by LPA acyltransferase (SLC1) to phosphatidic acid (PA). This is followed by dephosphorylation of PA by PA phosphohydrolase (PAP) to release diacylglycerol (DAG). In the final step, DAG is acylated either by DAG acyltransferase (DGA1 and DGA2 with acyl-CoA as an acyl donor) or by phospholipid DAG acyltransferase (LRO1 with glycerophospholipids as an acyl donor) to produce TAG. (C) To block the degradation pathways; the beta-oxidation (C1) and the TAG remobilization (C2). Free fatty acids (FFA) are activated by fatty acyl-CoA synthetase. The activated acyl-CoA (of N carbon) enter the beta-oxidation corresponding to four reaction catalyzed by the acyl-CoA oxidases (*POX1* to *POX6*) then by the multifunctional enzyme having both the hydratase and the deshydrogenase activity (MFE1) and finally the thiolase realizing an acyl-CoA (of N-2 carbon) and an acetyl-CoA. TAG remobilization could be blocked by inactivation of the triacylglycerol lipases (TGL3 and TGL4).

the six genes improved lipid accumulation even more if both *POX* genes and *GUT2* deletion were combined (Beopoulos, *et al.*, 2008). Similar improvements were obtained also by combining *POX* or MFE deletion together with GPD over expression (Dulermo and Nicaud, 2011). However, in Δpox1-6 strain, we observed induction of TGL4 and TGL3, suggesting that depending on genetic background, induction of TAG remobilization could be induced even during the accumulation phase. Indeed; deletion of TAG remobilization increases TAG accumulation in *Y. lipolytica* (Dulermo, *et al.*, to be published).

Finally, the fours metabolic engineering steps are to modify the fatty acid profiles of the TAG accumulated (Fig. 2D). In *Y. lipolytica* they are only two fatty acid desaturases (Δ9 desaturase, *OLE1* and Δ12 desaturase, *FAD2*) allowing to accumulate about nearly 50% linoleic acid in define condition (Beopoulos, *et al.*, 2008). Significant level of GLA was

Table 2. The lipid content and productivity of general edible oil plants (O'Brien, 2008).

Oil	Lipid content (%wt)	Oil yiel (kg/ha/yr)	Price (US$/kg)
Oilseeds			
Rapseed	45	591–664	1,23
Peanut	50	1260–1400	2,26
Soybean	20	450–506	1,14
Sunflower	45	517–664	1,52
Tree fruits and kernels			
Coconut	50	731–979	0,89
Olive	15–35	101–292	3,58
Palm	50	3004–5006	0,82
Palm kernel	50	300–500	0,88

Note: indexmundi.com a Refers to the average price between Aug 2012–Feb 2013 (from www.indexmundi.com)

synthesized in *Y. lipolytica* from endogenous linoleic acid (LA) and oleic acid (OA) by over expression of *M. alpina* Δ12-desaturase and Δ 6-desaturase (Chuang, *et al.*, 2010). Recently, Dupont de Nemours have developed *Y. lipolytica* strains for the production of oil enriched in EPA (patent US2009/0093543A1) or ARA (patent US2013/0123361A1) by overexpression of homologous and heterologous desaturases and elongases from different microorganisms. The key steps for lipid accumulation and the effort in metabolic engineering by multi-gene approach have been reviewed recently (Liang and Jiang, 2013). Thus, demonstrating that yeasts could be a great platform for the production of lipid (Beopoulos and Nicaud, 2012) and that these oil from *Y. lipolytica* are safe for human and animal feeds (Groenewald, *et al.*, 2013).

3 Economics of microbial oils production

The single-cell oil production cost depends mainly upon the species chosen for cultivation, lipid concentration within cells and the concentration of cells produced.

The oil productivity of oleaginous microorganisms is commonly indicated as g/l/day (or kg/m^3/day) which differs from oil plants (kg/ha/yr), as shown in Tables 2–4. Therefore, the biomass and oil productivity of oleaginous microorganisms (Tabs. 3 and 4) are presented as kg/m^3/yr, based on 300 days of operation per year.

From the lipid content in Table 4, cultivation in autotrophic conditions gives a higher fluctuation in the lipid content than that in heterotrophic conditions, which is probably due to the variation in the level of sunlight. The maximum oil productivity of *Schizochytrium limacinum* microalgae was 525.1 kg/m^3/yr, obtained by cultivation under a heterotrophic condition. Cultivation of *S. limacinum* in fermenter with a total volume of 10 m^3 yields similar lipid productivity as one hectare of oil palm.

Besides microalgae, many yeasts and fungi species are able to generate and accumulate lipids in their cell. The lipid content and productivity of selected oleaginous fungi and yeasts are presented in Table 3.

Table 3. The productivity of selected oleaginous yeasts, fungi and bacteria (Sawangkeaw, *et al.*, 2013).

Yeasts, fungi or bacteria strains	Lipid content (% w/w)	Productivity (kg/m3/yr)	
		Biomass	Lipid
Yeasts			
Candida curvata	29–58	691	315
Cryptococcus albidus	33–60	252	146
Cryptococcus curvatus	25–46	1990	1154
Lipomyces starkeyi	61–68	636	410
Rhodosporidium toruloides	58–68	3362	2120
Fungi			
Mucor mucedo	62		
Aspergillus oryzae	18–57	377	215
Cunninghamella echinulata	35–58	232	134
Mortierella isabellina	50–55	1276	679
Bacteria			
Arthrobacter sp.	>40	N/R	N/R
Acinetobacter calcoaceticus	27–38	N/R	N/R
Rhodococcus opacus	24–26	N/R	N/R
Bacillus alcalophilus	18–24	N/R	N/R

N/R = not reported.

Table 4. The lipid content, biomass and oil productivity of selected microalgae (Sawangkeaw, *et al.*, 2013).

Microalgae strain	Culture conditions	Lipid content (% w/w)	Productivity (kg/m3/yr)	
			Biomass	Lipid
Chlorella sp.	AT	22–32	159	54
Scenedesmus obliquus	AT,MT	13–58	153	54
Chaetoceros muelleri	AT	25–52	150	57
Chlorella zofingiensis	HT	52	216	112
Crypthecodinium Cohnii	HT	23	672	134
Nannochloropsis oculata	AT	23	870	200
Chlorella protothecoides	HT	48–64	412	231
Chaetoceros gracilis	AT	15–60	1065	404
Schizochytrium mangrovei	HT	68*	732	498
Schizochytrium limacinum	HT	50*	1044	525

*As total fatty acid content. AT, MT and HT are autotrophic, mixotrophic and heterotrophic cultivations, respectively.

Even with this high level of productivity, microbial oils are, for instance, too expensive to develop applications of specialty products (lubricants, polymers) or commodity product (biofuels). This explains the focus of actual industrial developments on the high value products as the microbial oils enriched in omega-3 and omega-6 for dietary supplements and for infant nutrition.

The cost of feedstock or carbon source required for the production of microbial lipids accounts for 60 to 75% of the total costs of the microbial oil. Hence, the economics of single-cell oil production can be improved by using low-cost and effective alternative feedstocks such as wastewater, municipal, and other carbonaceous industrial wastes and CO_2 in flue gases from boilers and power plants. Most of these substrates are locally available and thus are expected to support mainly small production facilities.

For example, several strains of yeast exhibit significant lipid production and accumulation levels with different substrates, such as sugarcane molasses (Cheirsilp, *et al.*, 2011) or wheat straw (Zheng, *et al.*, 2012).

Moreover, some oleaginous filamentous fungi can also produce lipids by utilizing raw glycerol, co-product of the biodiesel production (Chatzifragkou, *et al.*, 2011) sugarcane molasses, soluble starch and wheat straw (Hui, *et al.*, 2010).

The carbon sources obtained from lignocellulosic or sugar-based biomasses are one of the most interesting substrates for yeast and fungi cultivation. The conversion of lignocellulosic biomasses to lipid-based biomasses by cultivation with oleaginous yeasts, fungi or bacteria can utilize many agriculture, livestock and food and house hold wastes as feedstocks for lipid production. When the hydrolysate of rice straw or wheat straw obtained from dilute H_2SO_4 pretreatment was investigated for use as a substrate for the oleaginous yeasts *T. fermentans* and *Aspergillus oryzae*, respectively, the lipid accumulation level was reduced in presence of acetic acid, furfural,5-hydroxymethyl furfural and acid soluble lignin (Huang, *et al.*, 2009; Hui, *et al.*, 2010). However, the removal (detoxification) of such from the wheat straw hydrolysate enhanced the lipid production by *A. oryzae* (Hui, *et al.*, 2010).

Moreover, some fungi species, such as members from *Ephalosporium* or *Sclerocystis*, simultaneously accumulate lipid and produce cellulase (Peng, *et al.*, 2007). Due to the presence of cellulase, those fungi are able to digest amorphous cellulose in the lignocellulosic biomass to glucose and then anabolise this into a lipid-based biomass. Others works are focus on the screening of oleaginous yeast strains tolerant to lignocellulose degradation (Cheng, *et al.*, 2009) and evolutionary engineering of yeast. Therefore, oleaginous yeasts and fungi could be a possible link between lipid production and sugar-based biomasses.

Conclusion

Oleaginous microorganisms are alternative lipid-based biomasses that have a high potential, especially in terms of their productivity and a fast growth rate. Compared with edible and non-edible oil plant farming, the independence of climate provides a remarkable advantage for the cultivation of microorganisms in a closed system. However, the cost of microbial lipids is still too high and limits the application of such oleaginous microorganisms used for high value products. In order to develop applications of specialty products or commodity products, cheap carbon sources have necessarily to be used as carbon sources for the cultivation of the microorganisms and the performances of the bioprocess has to be further improved in terms of both the yield and the productivity. The exploration of the natural biodiversity is a promising strategy to identify novel oleaginous species or as enzymatic activity sources to engineer existing oleaginous species that assimilate and get lipids from agro-industrial residues or lingo-cellulosic biomass.

Further approaches combining genomics, transcriptomics, metabolomics and lipidomics techniques will undoubtedly provides deeper information of lipid production by oleaginous microorganism in order to optimize fatty acid profiles (chain length, unsaturation, functional group modifications) and to improve the use of low-cost raw material and productivity.

References

Akhtar P, Gray JI, Asghar A. 1998. Synthesis of lipids by certain yeast strains grown on whey permeate. *J. Food Lipids* 5: 283–297.

Aki T, Nagahata Y, Ishihara K, Tanaka Y, Morinaga T, Higashiyama K, Akimoto K, Fugikawa S, Kawamoto S, Shigeta S, Ono K, Suzuki O. 2001. Production of arachidonic acid by filamentous fungus. *J. Am. Oil Chem. Soc.* 78: 599–604.

Beopoulos A, Haddouche R, Kabran P, Dulermo T, Chardot T, Nicaud JM. 2012. Identification and characterization of a DAG acyltransferase belonging to the DGAT1 acyl-CoA:diacylglycerol acyltransferase family in the oleaginous yeast *Yarrowia lipolytica*. A new insight in storage lipid metabolism of oleaginous yeasts. *Appl. Microbiol. Biotechnol.* 93: 1523–1537.

Beopoulos A, Nicaud J-M, Gaillardin, C. 2011. Mini Review: An overview of lipid metabolism in yeasts and its impact on biotechnological processes. *Appl. Microbiol. Biotechnol.* 90: 1193–1206.

Beopoulos A, Nicaud JM. 2012. Yeast: A new oil producer. *OCL* 19: 22–88.

Chatzifragkou A, Makri A, Belka A *et al.* 2011. Biotechnological conversions of biodiesel derived waste glycerol by yeast and fungal species. *Energy* 36: 1097–1108.

Cheirsilp B, Suwannarat W, Niyomdecha R. 2011. Mixed culture of oleaginous yeast *Rhodotorula glutinis* and microalgae *Chlorella vulgaris* for lipid production from industrial wastes and its use as biodiesel feedstock. *New Biotechnol.* 28:362–368.

Chuang LT, Chen DC, Nicaud JM, Madzak C, Chen YH, Huang YS. 2010. Co-expression of heterologous desaturase genes in *Yarrowia lipolytica*. *New Biotechnol.* 27: 277–282.

Chuang LT, Chen DC, Chen YH, Nicaud JM, Madzak C, Huang YS. 2009. Production of functional g linoleic acid (GLA) by expression of fungal D12 and D6 desaturases genes in the oleagenous yeast *Yarrowia lipolytica*. In: Hou CT, Shaw J-F eds. Biocatalysis and Agricultural Biotechnology. London NY: PP. 163-180 CRC press.

Dulermo T, Nicaud J-M. 2011. Involvement of G3P shuttle and β-oxidation pathway into the control of TAG synthesis and lipid accumulation in *Yarrowia lipolytica*. *Metab. Eng.* 13: 482–491.

Dyal SD, Narine SS. 2005. Implications for the use of *Mortierella fungi* in the industrial production of essential fatty acids. *Food Res. Int.* 38: 445–467.

Easterling ER, French WT, Hernandez R, Licha M. 2009. The effect of glycerol as a sole and secondary substrate on the growth and fatty acid composition of *Rhodotorula glutinis*. *Bioresour. Technol.* 100: 356–361

Eroshin VK, Satroutdinov AD, Dedyukhina EG, Chistyakova TI. 2000. Arachidonic acid production by *Mortierella alpine* with growth-coupled lipid synthesis. *Proc. Chem.* 35: 1171–1175

Fakas S, Papanikolaou S, Batsos A, Panayotou MG, Malloucho A, Aggelis G. 2009. Evaluating renewable carbon sources as substrates for single cell oil production by *Cunninghamella echinulata* and *Mortierella isabellina*. *Biomass Bioenergy* 33: 573–580

Fakas S, Makri A, Mavromati M, Tselepi M, Aggelis G. 2009. Fatty acid composition in lipid fractions lengthwise the mycelium of *Mortierella isabellina* and lipid production by solid state fermentation. *Bioresour. Technol.* 100: 6118–6120.

Granger LM, Perlot P, Goma G, Pareilleux, A. 1993. Effect of various nutrient limitations on fatty acid production by *Rhodotorula glutinis*. *Appl. Microbiol. Biotechnol.* 38: 784–789.

Groenewald M, Boekhout T, Neuvéglise C, Gaillardin C, van Dijck PW, Wyss M. 2013. *Yarrowia lipolytica*: Safety assessment of an oleaginous yeast with a great industrial potential. *Crit. Rev. Microbiol.*, DOI:10.3109/1040841X.2013.770386.

Holdsworth JE, Veenhuis M, Ratledge C. 1988. Enzyme activities in oleaginous yeasts accumulating and utilizing exogenous or endogenous lipids. *J. Gen. Microbiol.* 134: 2907–2915.

Huang C, Zong M-H, Wu H, Liu QP. 2009. Microbial oil production from rice straw hydrolysate by *Trichosporon fermentans*. *Bioresour. Technol.* 100: 4535–4538.

Hui L, Wan C, Hai-tao D, Xue-jiao C Qi-fa Z, Yu-hua Z. 2010. Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid-state fermentation. *Bioresour. Technol.* 101: 7556–7562.

Kalscheuer R, Stolting T, Steinbuchel A. 2006. Microdiesel: *Escherichia coli* engineered for fuel production. *Microbiology* 152: 2529–2536

Liang M-H, Jiang J-G. 2013. Advancing oleaginous microorganisms to produce lipid via metabolic engineering technology. *Prog. Lipid Res.* 52: 395–408.

Miao XL, Wu QY. 2004. Bio-oil fuel production from microalgae after heterotrophic growth. *Renew. Energy.* 4: 41–44.

O'Brien RD. 2008. Fats and oils: formulating and processing for applications, 3rd ed. CRC Press.

Peng X-W, Chen H-Z. 2007. Microbial oil accumulation and cellulose secretion of the endophytic fungi from oleaginous plants. *Ann. Microbiol.* 57: 239–242.

Sawangkeaw R, Ngamprasertsith S. 2013. A review of lipid-based biomasses as feedstocks for biofuels production, *Renew. Sust. Energy. Rev.* 25: 97–108.

Steen EJ, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, del Cardayre SB, Keasling JD. 2010. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. *Nature* 463: 559–562.

Tai M, Stephanopoulos G. 2013. Engineering the push and pull of lipid biosynthesis in oleaginous yeast *Yarrowia lipolytica* for biofuel production. *Metab. Eng.* 15: 1–9.

Zhang X, Agrawal A, San K-Y, 2012. Improving fatty acid production in *Escherichia coli* through the overexpression of malonyl coA–acyl carrier protein transacylase. *Biotechnol. Prog.* 28: 60–65.

Zheng Y, Yu X, Zeng J, Chen S. 2012. Feasibility of filamentous fungi for biofuel production using hydrolysate from dilute sulfuric acid pretreatment of wheat straw. *Biotechnology Biofuels* 5: 1754–6834.

Zhu LY, Zong MH, Wu H. 2008. Efficient lipid production with *Trichosporon fermentans* and its use for biodiesel preparation. *Bioresour. Technol.* 99: 7881–7885.

Cite this article as: France Thevenieau, Jean-Marc Nicaud. Microorganisms as sources of oils. OCL 2013, 20(6) D603.