REVIEW

Sources of microbial oils with emphasis to *Mortierella* (*Umbelopsis*) *isabellina* fungus

Seraphim Papanikolaou[1] · George Aggelis[2]

Abstract

The last years a constantly rising number of publications have appeared in the literature in relation to the production of oils and fats deriving from microbial sources (the "single cell oils"—SCOs). SCOs can be used as precursors for the synthesis of lipid-based biofuels or employed as substitutes of expensive oils rarely found in the plant or animal kingdom. In the present review-article, aspects concerning SCOs (economics, biochemistry, substrates, technology, scale-up), with emphasis on the potential of *Mortierella isabellina* were presented. Fats and hydrophilic substrates have been used as carbon sources for cultivating Zygomycetes. Among them, wild-type *M. isabellina* strains have been reported as excellent SCO-producers, with conversion yields on sugar consumed and lipid in DCW values reported comparable to the maximum ones achieved for genetically engineered SCO-producing strains. Lipids produced on glucose contain γ-linolenic acid (GLA), a polyunsaturated fatty acid (PUFA) of high dietary and pharmaceutical importance, though in low concentrations. Nevertheless, due to their abundance in oleic acid, these lipids are perfect precursors for the synthesis of 2nd generation biodiesel, while GLA can be recovered and directed to other usages. Genetic engineering focusing on over-expression of $\Delta 6$ and $\Delta 12$ desaturases and of C16 elongase may improve the fatty acid composition (viz. increasing the concentration of GLA or other nutritionally important PUFAs) of these lipids.

Keywords *Mortierella isabellina* · Zygomycetes · Single cell oil

Single cell oils: general assessment of their potential

The last years there has been a rising upsurge of interest in the scientific literature related to the production of oils and fats from various microbial sources. These lipids, called also "single cell oils" (SCOs), are synthesized by the oleaginous microorganisms (Ratledge 1994; Ratledge and Wynn 2002), which are capable to produce storage lipid in significant quantities (i.e. > 20% in dry cell weight—DCW) inside their cells (Ratledge 1987, 1994; Papanikolaou and Aggelis 2011a; Bellou et al. 2016; Athenaki et al. 2018; Carsanba et al. 2018).

Attempts dealing with the production and utilization of SCO as alternative to commodity oils and fats have been made throughout most of the twentieth century. The most important efforts had been carried out in Germany during both world wars, with ultimate aim to develop economically viable processes that would provide high quantities of edible oils and fats for this country that, due to the blockade developed by the Allies, did not have access to major supplies of such types of commodities (Ratledge 1994; Ratledge and Wynn 2002). With the end of World War II, there has also been interest in other countries and principally the U.S. and the U.K. in relation to the production of SCOs (Woodbine 1959). However, the considerable developments in the agricultural practice that occurred after the early 1950s, meant, in fact, that common oils deriving from microbial sources (i.e. lipids rich in oleic, linoleic and α-linolenic acids) would have never been able to compete in terms of price, with the

✉ Seraphim Papanikolaou
spapanik@aua.gr

George Aggelis
George.Aggelis@upatras.gr

1   Laboratory of Food Microbiology and Biotechnology, Department of Food Science and Human Nutrition, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece

2   Unit of Microbiology, Department of Biology, Division of Genetics, Cell Biology and Development, University of Patras, 26504 Patras, Greece

bulk commodity oils, such as soybean oil, sunflower oil and rapeseed oil (Ratledge and Wynn 2002). Several studies suggest that the production cost of SCOs is high compared to that of the common oils and fats, principally due to the low productivity of oleaginous microorganisms in relation to the high cost of the fermentation (see techno-economic evaluations of the process in: Davies 1988; Koutinas et al. 2014a). However, the alternative option for large-scale production of SCOs still exists, since the price of oils, depending on their fatty acid (FA) composition, can present enormous variations ranging between 0.3 and 100 US $ per kg (Ratledge and Wynn 2002; Papanikolaou and Aggelis 2011a). Therefore, important aspects on the biotechnology of oils and fats refer to: (1) the isolation and identification of oleaginous microorganisms that are capable to produce in high amounts lipids presenting interesting structure and composition; (2) the optimization of culture conditions and the development of innovative fermentation strategies for improving lipid production; (3) the simultaneous production of microbial compounds produced through non-competitive pathways (i.e. enzymes, bio-colorants, etc.) for increasing sustainability of the process; (4) the combination (if possible) of various wastes and by-products as carbon sources in order to overcome the problem of reduced/seasonal substrate availability; (5) the production of SCOs in pilot-scale operations that allow for an integrated techno-economic evaluation of the process (Papanikolaou and Aggelis 2011a, b; Ratledge 2013a; Koutinas et al. 2014b; Bellou et al. 2016; Diwan et al. 2018). Interesting SCOs include cocoa-butter substitutes produced by a restricted number of oleaginous yeasts such as strains belonging to *Candida* sp., *Yarrowia lipolytica, Cryptococcus curvatus*, etc. (Papanikolaou and Aggelis 2010, 2011a, b; Athenaki et al. 2018; Carsanba et al. 2018) and those containing functional poly-unsaturated fatty acids (PUFAs) not frequently found in common oils, like *γ*-linolenic acid (GLA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), etc., synthesized by oleaginous fungi and micro-algae, mostly belonging to Zygomycetes, Peridiniales and Thraustochytrids (Ratledge and Wynn 2002; Bellou et al. 2014, 2016; Athenaki et al. 2018).
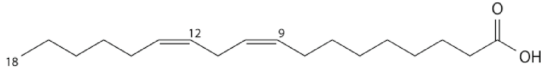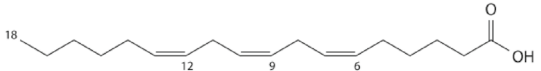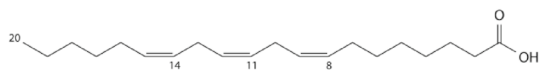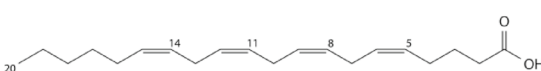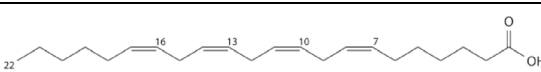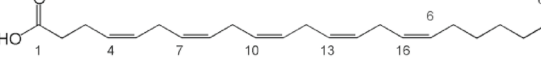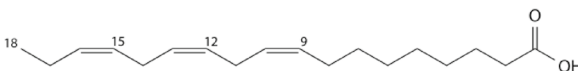
The last years there has been a continuously increasing utilization of the "1st generation" lipid-based biofuels, i.e. mostly biodiesel and, to lesser extent, renewable diesel, the production process of which is based on the utilization of various oils and fats as starting materials (Szczęsna-Antczak et al. 2006a; Papanikolaou and Aggelis 2009, 2011b). It has been claimed that utilization of edible common oils as starting materials amenable to be converted into biodiesel has resulted in an increase of the price of several conventional plant oils (Ratledge and Cohen 2008). In any case, and regardless of the accuracy or not of the above-mentioned assumption, the idea to convert, through oleaginous microorganisms, waste materials into lipid, that would further be

transformed into "2nd generation" biodiesel is remarkably attractive, since several problems (i.e. seasonal lipid availability, dependence from the climate, etc) would have been solved, while simultaneously sustainable, eco-friendly and "green" processes related with the valorization of residues would have been presented. Therefore, the last years there has been a rising necessity for the discovery of non-conventional sources of oils and fats, which could subsequently be converted into biodiesel (Szczęsna-Antczak et al. 2006a; Koutinas et al. 2014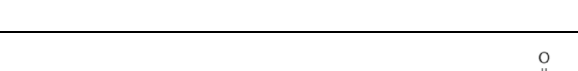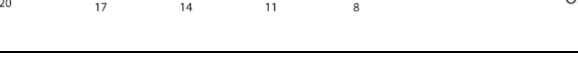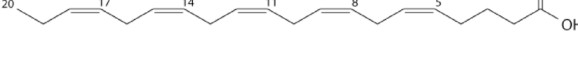b). The oleaginous microorganisms (mostly yeasts and fungi) cultivated on various low- or negative acquisition cost substrates are considered as candidates for the production of these lipids that would be converted into "2nd generation" lipid-based biofuels (Huang et al. 2013; Meeuwse et al. 2013; Patel et al. 2016, 2017; Qin et al. 2017; Diwan et al. 2018).

Substrates employed for the production of 2nd generation biodiesel include but are not limited to low-cost sugars (such as lignocellulosic sugars, sucrose and lactose or hydrolysates and wastewaters containing these sugars), biodiesel-derived glycerol and low-molecular weight organic acids or wastewaters containing in variable quantities the above-mentioned acids (Huang et al. 2013; Qin et al. 2017; Diwan et al. 2018). Obviously, fatty materials are not included in these substrates (Athenaki et al. 2018; Dourou et al. 2018). Hydrophobic materials though may be employed as substrates of oleaginous microorganisms, and this approach, has two axes of scientific and industrial interest: (1) the production of lipids that present economic or medical interest through biomodification of the employed fatty substrates. Typical examples include the production of cocoa-butter substitutes from low-cost industrial fats using the yeast *Yarrowia lipolytica* (Papanikolaou et al. 2001; Xiong et al. 2015; Daskalaki et al. 2018; Vasiliadou et al. 2018), or the synthesis of SCOs containing "bioactive" FAs like the GLA, during growth of several types of fungi on low-cost lipids (Aggelis et al. 1988, 1995a, b; Roux-Van der Merwe et al. 2005); (2) the production of microbial mass rich in lipids which may be employed as feed supplement, provided that the implicated microorganisms are of "GRAS"-type (Papanikolaou and Aggelis 2010, 2011b; Bellou et al. 2014, 2016).

Various fungi, fungus-like and micro-algal species have been used in order to produce lipids rich in PUFAs of medical and dietetic interest, such as GLA ($^{\Delta6,9,12}$C18:3), dihomo-*γ*-linolenic acid (DHGLA, $^{\Delta8,11,14}$C20:3), arachidonic acid (ARA, $^{\Delta5,8,11,14}$C20:4), DHA ($^{\Delta4,7,10,13,1619}$C22:6) and EPA ($^{\Delta5,8,11,14,17}$C20:5) (Čertik and Shimizu 1999; Ratledge and Wynn 2002; Dyal and Narine 2005; Wynn and Ratledge 2005; Sakuradani and Shimizu 2009; Ratledge 2013a; Bellou et al. 2014). PUFAs are functional FAs which include in their aliphatic chains two or more -*Z* (-*cis*) double bonds separated from each other by a single methylene group (Ratledge 1987). Oil accumulated by oleaginous fungi and

Table 1 The n-6 and n-3 families of polyene acids. (Adapted from Čertik and Shimizu 1999; Ratledge and Wynn 2002)

Common Name	Lipid name	Chemical name	Chemical structure
n-6 family			
Linoleic acid	18:2 (n-6)	all-cis-9,12-octadecadienoic acid	
γ-linolenic acid (GLA)	18:3 (n-6)	all-cis-6,9,12-octadecatrienoic acid	
Dihomo-γ-linolenic acid (DHGLA)	20:3 (n-6)	all-cis-8,11,14-eicosatrienoic acid	
Arachidonic acid (ARA)	20:4 (n-6)	all-cis-5,8,11,14-eicosatetraenoic acid	
Adrenic acid	22:2 (n-6)	all-cis-7,10,13,16-docosatetraenoic acid	
Docosapentaenoic acid	22:5 (n-6)	all-cis-4,7,10,13,16-docosapentaenoic acid	
n-3 family			
α-linolenic acid (ALA)	18:3 (n-3)	all-cis-9,12,15-octadecatrienoic acid	
Stearidonic acid (SDA)	18:4 (n-3)	all-cis-6,9,12,15-octadecatraenoic acid	
Eicosatetraenoic acid (ETA)	20:4 (n-3)	all-cis-8,11,14,17-eicosateetraenoic acid	
Eicosapentaenoic acid (EPA)	20:5 (n-3)	all-cis-5,8,11,14,17-eicosapentaenoic acid	
Docosahexaenoic acid (DHA)	22:6 (n-3)	all-cis-4,7,10,13,16,19-docosahexaenoic acid	

micro-algae is more unsaturated than that of the oleaginous yeasts, due to the higher capability of the former microorganisms to possess into their enzymatic battery the enzymes relevant to the synthesis of the various previously mentioned bioactive PUFAs (i.e. Δ4-, Δ5-, Δ6-, Δ12-, Δ15- and Δ17-desaturases, fatty acid elongases, etc.—Čertik and Shimizu 1999; Ratledge and Wynn 2002). Thus, oleaginous fungal, fungus-like and micro-algal species have been principally used in order to produce lipids rich in medically and nutritionally important PUFAs, while few yeast strains have been recorded to be capable to synthesize such types of PUFAs (Dyal and Narine 2005; Sakuradani and Shimizu 2009;

Papanikolaou and Aggelis 2010). Microbial lipids are mostly stored in the form of triaclyglycerols (TAGs) (Ratledge and Wynn 2002; Bellou et al. 2016; Athenaki et al. 2018). Other components presented in non-negligible quantities are free FAs, other neutral lipids (such as monoacylglycerols, diacylglycerols and steryl-esters), sterols and polar fractions (e.g. phospholipids, sphingolipids, glycolipids) (Bellou et al. 2012, 2014; Gardeli et al. 2017; Athenaki et al. 2018). The two most common PUFA families, namely omega-3 (ω-3/n-3) and omega-6 (ω-6/n-6), are detailed in Table 1 (Čertik and Shimizu 1999). These two families are diversified each other by the fact of the introduction of the first double bond starting from the -CH_3 terminus, that is the 3rd carbon (for the case of the n-3 family) or the 6th carbon (for the case of the n-6 family) (Čertik and Shimizu 1999).

Large-scale SCO production operations have been reported in New Zealand at the end of 1980s, where *Apiotrichum curvatum* (= *C. curvatus*) ATCC 20509 growing on cheese-whey in pilot-scale operations (viz. 500-L bioreactors) was used (Davies 1988; Davies and Holdsworth 1992). Following techno-economic evaluation of the process, the price of the produced SCO was *c*. 1.0 US $ per kg (Davies 1988). Earlier, within the premises of "Fuji Oil Co. Ltd." (Osaka, Japan), Tatsumi et al. (1977) have performed batch cultures of the yeast *Rhodosporidium toruloides* IFO 0413 on glucose-based media in semi-pilot-scale operations (viz. 150-L bioreactor with active volume of 100 L). Yeast dry biomass (*c*. 13 g/L containing 59.8% of lipids in DCW) was obtained, and after solvent extract and de-acidification of the microbial lipid, recovery of the more saturated microbial lipid fraction was carried out. This saturated fraction partially substituted cocoa-butter, and the resultant chocolate that was produced presented better technical and almost equivalent organoleptic characteristics with the typical control chocolate that had been produced without added microbial lipid (Tatsumi et al. 1977).

GLA-containing SCO production in semi-industrial level was carried out in the U.K. at the late 1980s, with the use of *Mucor circinelloides* cultivated in 220 m^3 bioreactors (Ratledge 1987, 2013a). Currently "Martek Biosciences Corp." produces in large-scale operations DHA-rich SCO, while ARA-rich SCO is produced by the companies "Dutch State Mines", "Suntory" and "Cargill" in conjunction with "Wuhan Alking Bioengineering Co. Ltd" (Ratledge 2013a). Concerning biodiesel, "Sapphire Energy Inc." (New Mexico, US) has established a plant aiming at producing biodiesel with the target of 10,000 barrels per day by the end 2018 using photosynthetically grown algae in raceway ponds (Ratledge 2013b). "Neste Oyj" (until 1 July 2015 "Neste Oil Corporation") (Porvoo—Finland) has inaugurated a couple of years ago its first pilot for yeast-lipid production amenable for biodiesel synthesis from purified lignocellulosic sugars (Athenaki et al. 2018).

In the large-scale SCO production operations mentioned above, sugars and related substrates or CO_2 have been employed as substrates (Ratledge 1987, 2013a). Medium-scale operations in which fatty materials had been employed as substrates (i.e. methyl-stearate, methyl-palmitate, vinyl-stearate, vinyl-palmitate, ethyl-stearate, etc.) have been carried out within the premises of "Fuji Oil Co. Ltd." (Osaka, Japan), and yeast lipids that presented composition and structure similarities with the cocoa-butter have been produced using *Candida* sp. ATCC 20504, *C. guilliermondii* IFO 0838, *C. tropicalis* OUT 6019, *Trichosporon* sp. ATCC 20505, *Trichosporon* sp. ATCC 20506 and *Torulopsis versatilis* OUT 6204 (Matsuo et al. 1981).

While techno-economic evaluations of the typical sugar-to-oil conversions have been made (Davies 1988; Koutinas et al. 2014a) demonstrating that the principal cost of the process is the one of the fermentation, the corresponding evaluation of the oil-to-oil conversion has not been carried out yet. However, the oil-to-oil conversion performed by oleaginous yeasts can present industrial interest in the near future due to the very high conversion yield that can be = 0.35–0.50 g/g and in several cases > 0.6 g/g (Matsuo et al. 1981; Papanikolaou et al. 2001, 2002, 2007a, 2011; Vasiliadou et al. 2018), while in the sugar-to-oil conversion this conversion yield is rarely > 0.20 g/g (Ratledge and Wynn 2002; Ratledge and Cohen 2008). Fatty materials of zero or negative acquisition cost (Papanikolaou et al. 2007a, 2011) can be employed as substrates in the process while tailor made expensive SCOs production that is of industrial importance, with very satisfactory conversion yields, can occur.

Single cell oils: characteristics, substrates used and biochemistry of their synthesis— potential of *M. isabellina*

Introductory elements on Zygomycetes and *M. isabellina* oils

Strains of the Zygomycete fungus *M. isabellina* (synonyms for the genus: *Actinomortierella, Carnoya, Naumoviella, Haplosporangium, Azygozygum* and recently *Umbelopsis*; see: Meeuwse et al. 2011a; Wagner et al. 2013; Takeda et al. 2014; Dourou et al. 2017) have been revealed capable of producing considerable amount of lipids varying between 75 and 84% w/w in DCW (Chatzifragkou et al. 2010; Papanikolaou et al. 2017) during growth on several low-cost substrates such as commercial sugars, molasses, biodiesel derived glycerol, lignocellulosic sugars and hydrolysates, plant oils, etc. (Bellou et al. 2016; Athenaki et al. 2018).

Zygomycetes include oleaginous species that are potential producers of SCO containing GLA, a PUFA of crucial dietary and pharmaceutical importance (Ratledge 1994,

2013a; Ratledge and Wynn 2002; Dyal and Narine 2005; Bellou et al. 2014, 2016). The biosynthesis of GLA is a taxonomic characteristic for the Zygomycetes (Ratledge 1994), whereas only a restricted number of non-Zygomycetes fungi (i.e. some strains of *Aspergillus fumigatus* and *Emericella nidulans*—see i.e. Roux-Van der Merwe et al. 2005) has been revealed capable of producing lipids containing GLA. Lipids containing GLA are of high added-value for both the food and the pharmaceutical industries since they present anti-thrombotic, anti-irritant and especially anti-tumor properties, they are also effective in the treatment of various diseases and organic dysfunctionalities such as control (decrease) of blood pressure, inflammatory disorders, multiple sclerosis, premenstrual tension (in women), rheumatoid arthritis and atopic eczema whereas they are implicated in the production of immune-regulating eicosanoïds and they contribute positively in the function of immune cells (Ratledge 1994, 2013a; Ratledge and Wynn 2002; Barre 2009; Bellou et al. 2016). *Cunninghamella echinulata* and *Thamnidium elegans* SCOs (containing GLA), in the form of FA lithium or potassium salts (FAPS/FALS) present interesting anti-cancer activities against several cancer cell lines (e.g. HL-60 and MCF-7 cell lines) (Alakhras et al. 2015; Sayegh et al. 2016). Vegetable oils, such as the evening primrose oil (EPO) and the borage oil (BO), which contain this FA in various concentrations ranging from 8 to 22%, are, in general, quite expensive; these lipids (EPO contains GLA in concentrations 8–10% w/w of total lipids, and BO 20–22% w/w of total lipids), have an elevated cost estimated to > 20 US $ per kg (Ratledge and Wynn 2002; Papanikolaou et al. 2008; Barre 2009). Thus, SCO containing GLA production presents significant potential (Bellou et al. 2016; Athenaki et al. 2018). On the other hand, due to the abundance of oleic acid ($^{\Delta 9}$C18:1) found into the lipids of many Zygomycetes and mostly *M. isabellina*, these lipids could constitute perfect precursors for 2nd generation biodiesel, while the precious GLA can previously be recovered and used in the pharmaceutical and chemical industry. In fact, the requirement for making a satisfactory biodiesel is that the TAGs amenable to be converted into biodiesel should be rich in saturated and, mostly, monounsaturated FAs such as palmitic acid (C16:0), stearic acid (C18:0), and oleic acid ($^{\Delta 9}$C18:1) (Ratledge 2011), while a concentration of $^{\Delta 9}$C18:1 into the lipids ranging between 50 and 66% w/w, would result in the synthesis of a perfect-type biodiesel (Knothe 2005; Moser 2009). Unsaturated fatty acids, particularly the PUFA ones with three or more double bonds, are undesirable as they readily auto-oxidize making the final biodiesel technically unsatisfactory as well as giving it an unpleasant smell (Ratledge 2011).

In the next paragraphs, the biochemistry and technology of lipid production when hydrophobic and hydrophilic substrates are employed will be presented and critically discussed, with emphasis given on the potential of SCO production by Zygomycetes and mostly *M. isabellina*.

Hydrophobic compounds as substrates for oleaginous microorganisms and *M. isabellina*

Fatty materials have been used as microbial substrates for several oleaginous microorganisms (Papanikolaou and Aggelis 2010, 2011a, b; Athenaki et al. 2018; Carsanba et al. 2018). When hydrophobic materials are employed as substrates, the process of lipid accumulation (the so-called "ex novo" lipid production) is radically different to the one occurring when glucose or similarly catabolized compounds are employed as substrates (viz. the "de novo" lipid production) (Papanikolaou and Aggelis 2011a, b; Carsanba et al. 2018; Dourou et al. 2018). Specifically, when fats (mainly consisting of triacylglycerols—TAGs) are employed as carbon sources in microbial cultures, substrate TAGs are firstly hydrolyzed by the extra-cellular or cell bounded lipases to generate assimilable carbon compounds (i.e. FAs, glycerol). Oleaginous Zygomycetes like *Amylomyces rouxii, M. isabellina* and *M. circinelloides* have been revealed capable to synthesize various types of lipolytic enzymes in significant quantities, remarkably hydrolyzing the substrate TAGs and converting them into free FAs, diacylglycerols (DAGs), etc. (Koritala et al. 1987; Kendrick and Ratledge 1996; Weber and Tribe 2003; Szczęsna-Antczak et al. 2006b, 2018). TAG hydrolysis is a selective process since in most cases, microbial lipases exhibit important regio-selectivity and, in many cases, the substrate FAs that are esterified at the external positions of the TAGs are firstly released, while subsequently, the remaining β-monoacylglycerols are hydrolyzed following their isomerization to α-isomer (Metzger and Bornscheuer 2006; Szczęsna-Antczak et al. 2006a, b; Vasiliadou et al. 2018). Besides regio-selectivity, microbial lipases may also exhibit significant typo-selectivity, recognizing hence specific aliphatic chains according to their number of carbon atoms and the existing double bonds (Aggelis et al. 1995a, b, 1997; Fickers et al. 2005; Saygün et al. 2014). The various FAs, after having been released from the TAG structures, are incorporated into the microbial cells, usually with different incorporation rates depending on their structure and the sufficiency of cell membrane in specific FA carriers (Papanikolaou et al. 2001; Papanikolaou and Aggelis 2011a; Tzirita et al. 2018; Vasiliadou et al. 2018). Finally, the incorporated aliphatic chains are selectively oxidized in the microbial cell, usually through β-oxidation process, or used as building blocks for the cellular lipid biosynthesis or, finally, are subjected to modification reactions (i.e. dehydrogenation, elongation) (Papanikolaou and Aggelis 2011a, b). By considering all previously indicated biochemical events, it can be concluded that ex novo lipid production process is a growth-associated anabolic activity, that is

realized regardless of the presence of assimilable nitrogen into the growth medium (Papanikolaou and Aggelis 2010; Papanikolaou et al. 2007a, 2011; Vasiliadou et al. 2018). On the other hand, during the ex novo lipid production process some quantities of extra-cellular nitrogen need to be found into the growth medium in order to support sufficient production of microbial mass. However, if nitrogen is presented in excessively high concentrations resulting to low C/N molar ratios into the medium, the extra-cellular carbon flow is mostly directed towards the synthesis of lipid-free material (i.e. proteins), rather than to storage lipids (Bati et al. 1984; Papanikolaou et al. 2002, 2007a). In any case, during the ex novo lipid production process, lipid accumulation normally occurs independently to the presence of nitrogen into the culture medium, and this is in disagreement with the biochemical process of lipid accumulation conducted when sugars or similarly metabolized compounds are employed as substrates (Ratledge and Wynn 2002; Papanikolaou and Aggelis 2011a).

Zygomycetes like *M. isabellina, T. elegans, C. echinulata, C. elegans, A. rouxii, M. circinelloides, Conidiobolus nanodes, Gilbertella persicaria, Rhizopus oligosporus*, etc., have been revealed capable to consume fatty materials employed as sole substrate (or co-substrate with sugars) (Koritala et al. 1987; Aggelis et al. 1995a, b; Kendrick and Ratledge 1996; Čertik et al. 1997; Roux-Van der Merwe et al. 2005; Szczęsna-Antczak et al. 2006b, 2018). Some of the above species exhibit extensive hydrolytic activity rapidly after inoculation (Koritala et al. 1987; Szczęsna-Antczak et al. 2006b). Concerning the biomodification of fatty substrates performed by Zygomycetes, the available information is rather contradictory; while in several cases in which trials had been carried out with plant oils employed as substrate, SCO produced contained non-negligible quantities of GLA or long-chain PUFAs, indicating significant activity of the intra-cellular Δ6 desaturase and elongases (Aggelis et al. 1995b; Čertik et al. 1997; Roux-Van der Merwe et al. 2005; Szczęsna-Antczak et al. 2006b, 2018), in other cases growth on plant oils was accompanied by almost complete cessation of PUFAs synthesis, presumably due to almost complete inhibition of the above-mentioned enzymes performed by the substrate aliphatic chains found into the medium, and this was mainly reported for *M. isabellina* fungus (Kendrick and Ratledge 1996).

When fatty materials are employed as microbial substrates, the pathways that are involved in the assimilation process, include the following steps: (1) lipase-catalyzed hydrolysis of the TAGs or the alkyl-esters (i.e. methyl-esters) employed as microbial substrates; when free FAs are employed as substrate, the involvement of lipases is not mandatory; (2) incorporation of the released aliphatic chains inside the cells or mycelia with various incorporation rates depending on the FA composition of the fatty substrate employed; (3) reactions of biomodification of the FAs previously incorporated inside the microbial cells, like elongation and/or desaturation; as indicated by Kendrick and Ratledge (1996), the above-mentioned reactions may not occur, provided that in several cases the exogenous FAs provoke inhibition in the enzymes implicated in these reactions; (4) accumulation of storage lipid, viz. reactions of incorporation of FA chains inside the stored microbial TAGs; in several cases remarkable enhancement of lipid accumulation occurs when fatty materials are added as substrate, compared to growth on glucose employed as substrate (Kendrick and Ratledge 1996; Szczęsna-Antczak et al. 2006b, 2018; Papanikolaou et al. 2007a, 2008, 2011); (5) dissimilation of the acyl-CoA units, via β-oxidation pathway—in each cycle of the pathway, an acetyl-CoA unit and an acyl-CoA unit containing two atoms of carbon less than the initial aliphatic chain is generated; (6) biodegradation of the generated acetyl-CoA through the Krebs cycle (production of CO_2, ATP and energy); (7) anabolism of portion of the acetyl-CoAs generated after β-oxidation through the glyoxylate by-pass (reactions of glyconeogenesis or synthesis of amino-acids); (8) biodegradation ("turnover") of the previously stored TAGs when the extra-cellular carbon source is no longer available or its concentration is substantially low to cover the microbial metabolic requirements, yielding in the synthesis of acetyl-CoA, that will further be catabolized through Krebs cycle or will be used for anabolic reactions through the glyoxylate by-pass (Čertik et al. 1997; Papanikolaou et al. 2001, 2006; Papanikolaou and Aggelis 2011a; Athenaki et al. 2018; Tzirita et al. 2018). In the case in which blends of sugars (or similarly catabolized compounds i.e. glycerol) with fatty materials are employed as simultaneous substrates, in the above-mentioned pathways, another pathway, namely the one of the de novo intra-cellular FA and subsequently TAG synthesis from acetyl-CoA derived from sugar catabolism should also be considered (Kendrick and Ratledge 1996; Čertik et al. 1997; Papanikolaou et al. 2006; Papanikolaou and Aggelis 2011a). Concerning the later pathway, fatty acid synthase (FAS) and ATP-citrate lyase (ACL) (the main enzymes implicated in the de novo lipid production process) are strongly inhibited by the presence of exogenous aliphatic chains at least in the case of *Saccharomyces cerevisiae* (Meyer and Schweizer 1976), and, therefore, the presence of fatty materials in the culture medium should be generally incompatible with de novo biogenesis of FAs from glucose. However, some de novo lipid biosynthesis has been identified despite the significant presence of fatty materials into the medium for *M. isabellina* (Kendrick and Ratledge 1996) or other oleaginous Zygomycetes strains (i.e. *T. elegans, C. echinulata, C. elegans*) (Čertik et al. 1997). Likewise, other microorganisms like strains of the yeasts *Pichia methanolica* and *Y. lipolytica* have been revealed capable to perform some de novo fatty acid biosynthesis from glucose or glycerol, in
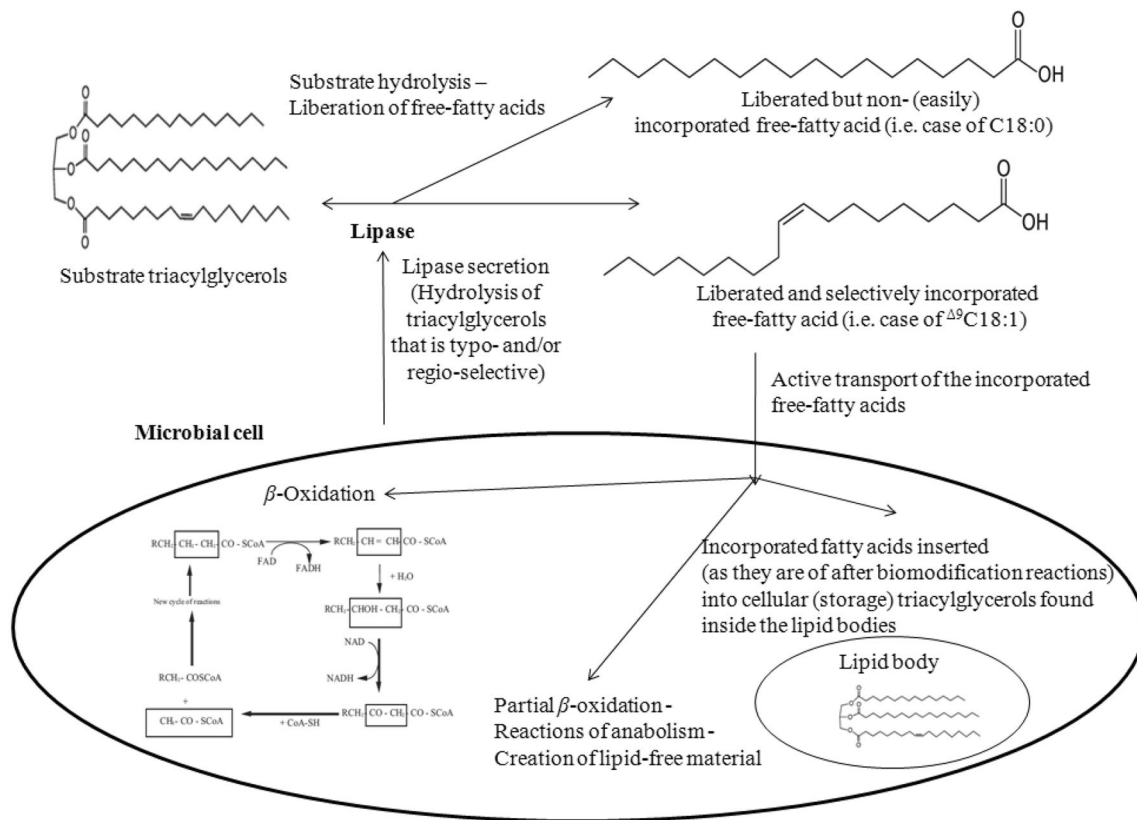
Substrate hydrolysis –
Liberation of free-fatty acids

Liberated but non- (easily)
incorporated free-fatty acid (i.e. case of C18:0)

Substrate triacylglycerols

Lipase

Lipase secretion
(Hydrolysis of
triacylglycerols
that is typo- and/or
regio-selective)

Liberated and selectively incorporated
free-fatty acid (i.e. case of $^{\Delta 9}$C18:1)

Active transport of the incorporated
free-fatty acids

Microbial cell

β-Oxidation

$RCH_2 \cdot CH_2 \cdot CH_2 \cdot CO \cdot SCoA$ $RCH_2 \cdot CH = CH \cdot CO \cdot SCoA$

FAD   FADH   + H$_2$O

New cycle of reactions

$RCH_2 \cdot CHOH \cdot CH_2 \cdot CO \cdot SCoA$

NAD

$RCH_2 \cdot COSCoA$ NADH

+

$CH_3 \cdot CO \cdot SCoA$ + CoA-SH $RCH_2 \cdot CO \cdot CH_2 \cdot CO \cdot SCoA$

Incorporated fatty acids inserted
(as they are of after biomodification reactions)
into cellular (storage) triacylglycerols found
inside the lipid bodies

Lipid body

Partial β-oxidation -
Reactions of anabolism -
Creation of lipid-free material

Fig. 1 Consecutive steps of biodegradation of fatty substrates by oleaginous microorganisms: illustration of enzyme-catalyzed breakdown of triacylglycerols and subsequent steps of incorporation and catabolism of the incorporated inside the microbial cells free fatty acids. It is indicated that unsaturated fatty acids (i.e. oleic acid) enter inside the cells of the oleaginous microorganisms, while saturated fatty acids (i.e. stearic acid) do not (easily) enter due to negative selectivity. Incorporated fatty acids will be partially or completely subjected to β-oxidation, or will enter inside the triacylglycerol structures of the cellular lipids. [Adapted from Papanikolaou and Aggelis (2010), Papanikolaou and Aggelis (2011a) and Vasiliadou et al. (2018)]

spite of the presence of exogenous aliphatic chains into the culture medium (Aoki et al. 2002; Papanikolaou et al. 2003, 2006), having as result the "tailoring" of the synthesis of intra-cellular lipids and, thus, the potential production of high-value cellular lipids (i.e. cocoa-butter substitutes) from blends of fatty agro-industrial residues with glucose and/or glycerol (Papanikolaou et al. 2003, 2006; Papanikolaou and Aggelis 2010).

The consecutive steps of the breakdown of fatty materials and the subsequent incorporation and catabolism of the released FAs, are illustrated in Fig. 1.

Results dealing with the cultivation of oleaginous Zygomycetes, with emphasis given on the potential of *M. isabellina* fungus, during growth on fatty materials employed as substrate (or co-substrate) are illustrated in Table 2.

Sugars and related compounds as substrates for oleaginous microorganisms and *M. isabellina*

Remarkable differences in biochemical and kinetic level exist between the processes of lipid accumulation from glucose or similarly metabolized compounds compared to that occurring from fatty materials; de novo lipid accumulation process in most cases is a non-growth associated process, conducted after nitrogen (and, to lesser extent, sulfate and/ or phosphorus) depletion from the culture medium. Nitrogen exhaustion leads to a rapid decrease of the concentration of cellular AMP, which is used in order for nitrogen deriving from this compound to be utilized by the rapidly nitrogen-depleted microbial cells. Cellular AMP concentration decrease results in a remarkable drop of the Krebs cycle function, since NAD$^+$- (and in various cases NADP$^+$-) isocitrate dehydrogenase, enzymes that are allosterically activated by AMP and ADP, lose their activity. Therefore, iso-citrate, and in succession citrate, is accumulated inside the mitochondria. When this concentration becomes higher

Table 2 Lipid production by *M. isabellina* or other oleaginous Zygomycetes growing on hydrophobic materials (or blends of hydrophobic and hydrophilic substances) under several cultivation configurations

Carbon source	Microorganism	DCW (g/L)	Lipid in DCW (%, w/w)	Cultivation mode	References
Zygomycetes					
Sunflower oil	*M. circinelloides*[a]	5.4	36.8	Shake flasks	Aggelis et al. ([1995a](#))
Trolein	*C. nanodes*[b]	10.8	44.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Linseed oil	*C. nanodes*[b]	12.3	42.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Triolein	*M. circinelloides*[a]	4.8	38.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Safflower oil	*M. circinelloides*[a]	6.4	35.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Sesame oil	*E. exitalis*[c]	8.8	36.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Safflower oil	*E. exitalis*[c]	7.1	25.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Sunflower oil and glucose	*M. mucedo*[a]	19.4	62.0	Shake flasks	Čertik et al. ([1997](#))
Sunflower oil and glucose	*M. plumbeus*[a]	19.8	60.0	Shake flasks	Čertik et al. ([1997](#))
Sunflower oil and glucose	*C. echinulata*[d]	18.3	58.0	Shake flasks	Čertik et al. ([1997](#))
Sunflower oil and glucose	*C. elegans*[d]	18.0	53.0	Shake flasks	Čertik et al. ([1997](#))
Sunflower oil and glucose	*T. elegans*[f]	18.6	56.0	Shake flasks	Čertik et al. ([1997](#))
Sunflower oil and glucose	*R. microscopus*[e]	18.2	43.0	Shake flasks	Čertik et al. ([1997](#))
Sunflower oil and glucose	*R. stolonifer*[e]	18.9	63.0	Shake flasks	Čertik et al. ([1997](#))
Edible-oil-containing waste (sunflower oil ~80% w/w)	*C. echinulata*[d]	24.9	24.0	Shake flasks	Roux-Van der Merwe et al. ([2005](#))
Olive oil	*M. circineloides*[a]	24.1	54.0	Shake flasks	Szczęsna-Antczak et al. ([2006b](#))
Olive oil and glucose	*M. circineloides*[a]	39.9	65.0	Shake flasks	Szczęsna-Antczak et al. ([2006b](#))
Olive oil	*M. racemosus*[a]	23.1	46.7	Shake flasks	Szczęsna-Antczak et al. ([2006b](#))
Olive oil and glucose	*M. racemosus*[a]	32.2	70.8	Shake flasks	Szczęsna-Antczak et al. ([2006b](#))
Fried soybean oil	*Rhizopus* sp.	10.1	28.4	Shake flasks	Tauk-Tornisielo et al. ([2009](#))
Canola oil	*Rhizopus* sp.	10.0	40.6	Shake flasks	Tauk-Tornisielo et al. ([2009](#))
Palm oil	*Rhizopus* sp.	11.9	54.3	Shake flasks	Tauk-Tornisielo et al. ([2009](#))
Sesame oil	*M. circinelloides*[a]	12.9	40.0	Shake flasks	Tauk-Tornisielo et al. ([2009](#))
Canola oil	*M. circinelloides*[a]	9.6	36.6	Shake flasks	Tauk-Tornisielo et al. ([2009](#))
Olive oil and glycerol	*M. circinelloides*[a]	33.8	47.3	Shake flasks	Szczęsna-Antczak et al. ([2018](#))
Olive oil	*M. circinelloides*[a]	31.4	47.8	Shake flasks	Szczęsna-Antczak et al. ([2018](#))
Technical fatty acids	*M. circinelloides*[a]	49.4	56.7	Shake flasks	Szczęsna-Antczak et al. ([2018](#))
Fatty acid ethyl esters	*M. circinelloides*[a]	41.2	49.8	Shake flasks	Szczęsna-Antczak et al. ([2018](#))
Lecithin from rape oil processing	*M. circinelloides*[a]	39.6	56.6	Shake flasks	Szczęsna-Antczak et al. ([2018](#))
Mortierella isabellina					
Triolein	Strain CBS 224.35	8.4	43.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Sesame oil	Strain CBS 224.35	6.9	46.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Safflower oil	Strain CBS 224.35	9.7	46.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Triolein and glucose	Strain CBS 224.35	9.2	33.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Sesame oil and glucose	Strain CBS 224.35	8.1	34.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Safflower oil and glucose	Strain CBS 224.35	8.4	31.0	Vortex-bottles	Kendrick and Ratledge ([1996](#))
Sunflower oil and glucose	Strain CCF-14	9.4	50.9	Shake flasks	Čertik et al. ([1997](#))
Sunflower oil and glucose	Strain CCF-1088	10.1	56.6	Shake flasks	Čertik et al. ([1997](#))
Sunflower oil and glucose	Strain CCF-1098	10.3	55.2	Shake flasks	Čertik et al. ([1997](#))

[a]*Mucor*

[b]*Conidiobolus*

[c]*Entomophtora*

[d]*Cunninghamella*

[e]*Rhizopus*

[f]*Thamnidium*

than a critical value, citric acid is secreted inside the cytoplasm in exchange with malate. Then, citric acid is cleaved by ACL, enzyme-key showing the oleaginous character of the microorganisms, into acetyl-CoA and oxaloacetate, and acetyl-CoA, by virtue of the action of FAS generates cellular fatty acids and subsequently TAGs, that are the most common form of lipophilic compounds found in the oleaginous microorganisms (Ratledge and Wynn 2002; Papanikolaou et al. 2004a; Papanikolaou and Aggelis 2011a; Dourou et al. 2018).

In the case of non-lipid producing microorganisms (therefore in the absence or poor activity of ACL), the microbial response upon nitrogen limitation is the (significant in several cases) secretion of low molecular weight compounds, mostly citric acid and, to lesser extent, mannitol and/or erythritol, into the medium (Papanikolaou and Aggelis 2011a; Athenaki et al. 2018; Dourou et al. 2018). As far as the production of citric acid is concerned, mostly yeasts of the species *Y. lipolytica* and fungi of the species *Aspergillus niger* are implicated, whereas, the poor regulation of ACL can biochemically explain the biosynthesis and accumulation into the culture medium of citric acid, since, as mentioned citric acid itself is the substrate of ACL action (Ratledge and Wynn 2002; Papanikolaou and Aggelis 2009). On the other hand, the synthesis of polyols as microbial response of nitrogen limitation imposed into the medium, has not yet been completely elucidated on biochemical and molecular level (Rywińska et al. 2013). Mannitol, in low quantities, has been produced together with significant storage lipid accumulation under nitrogen limitation by *M. isabellina* strains growing on glucose-based media under nitrogen limitation (Gardeli et al. 2017).

In another possibility in which poor or no regulation of ACL occurs, nitrogen depletion will theoretically result in the inhibition of the action of the enzyme 6-phospho-fructokinase (PFK), which equally is allosterically activated by the presence of generated AMP (Zhong and Tang 2004). In further association to the decreased activity of 6-phosphoglucose isomerase (PGI) (Philippoussis and Diamantopoulou 2012), intra-cellular accumulation of polysaccharides would occur as response of extra-cellular nitrogen limitation imposed (Ratledge 1994; Papanikolaou and Aggelis 2011a). However, this hypothesis seems to be incomplete, at least for the case of the oleaginous fungus *M. isabellina* (Dourou et al. 2017; Gardeli et al. 2017), and also for yeasts such as *Cryptococcus curvatus* and *Y. lipolytica* (Tchakouteu et al. 2015; Dourou et al. 2017); in the above-mentioned microorganisms it has been indicated that significant production of endopolysaccharides (i.e. > 30% w/w in DCW and in some cases up to *c*. 70% w/w) occurred in the balanced growth phase and at the early culture stages during which remarkable quantities of nitrogen were found into the medium. This significant accumulation of endopolysaccharides during the

balanced growth phase and in the presence of nitrogen into the medium, would potentially suggest saturation of glycolytic enzymes like PFK and PGI, and intra-cellular carbon diversion towards the synthesis of endopolysaccharides despite the nitrogen presence into the medium, in contrast to the considerations related with the accumulation of storage compounds by several types of microorganisms under nitrogen limitation (Ratledge and Wynn 2002). More specifically, studies on carbon metabolism in the non-conventional yeast *Y. lipolytica* and the fungus *M. isabellina* have revealed that during the balanced and the early oleaginous phases, polysaccharide accumulation is triggered as a result of insufficient enzymatic activity of PFK to drive the catabolism of hexoses towards pyruvate synthesis, while in the case of *M. isabellina* and during the lipid accumulation phase, cellular polysaccharides' degradation and reconstruction was also observed, as suggested by the high enzymatic activities of phosphoglucomutase (PGM) and fructose-1,6-biphosphatase (FBP) (Dourou et al. 2017). On the contrary, there is no evidence of such inter-conversion between lipids and polysaccharides during the lipid accumulation phase in *Y. lipolytica*. Finally, during cellular lipid degradation period, in both *Y. lipolytica* and *M. isabellina*, high activities of PGM, transaldolase (TALDO) and FBP enzymes, involved in polysaccharide biosynthesis, degradation and reconstruction are recorded (Dourou et al. 2017).

The previous analysis showed that, for at least some types of oleaginous microorganisms including strains of *M. isabellina, Y. lipolytica* and *C. curvatus*, during growth on sugars in the balanced growth phase, the extra-cellular carbon source was principally converted into endopolysaccharides. Interestingly, in the above-indicated cases, as fermentation proceeded endopolysaccharides together with the supplementary consumption of the extra-cellular sugar were converted into storage lipids (Tchakouteu et al. 2015; Dourou et al. 2017). This inter-play between the synthesis of intra-cellular total carbohydrates and microbial oils is a quite interesting biochemical event, and has also been reported for some other microorganisms like *Chlorella* sp. growing autotrophically under constant illumination conditions in open-pond simulating photo-bioreactors (Bellou and Aggelis 2012). The inverse trend has been reported for several higher fungi (i.e. *Pleurotus pulmonarius, Agrocybe aegerita, Ganoderma applanatum* and *Volvariella volvacea*) cultivated on glucose in static or agitated liquid cultures. These fungi during liquid cultures on glucose have shown elevated lipid accumulation at the beginning of their growth and despite the presence of nitrogen into the medium (i.e. lipid in DCW values in some cases ≥ 20% w/w have been reported although the above-mentioned microorganisms are not oleaginous). Lipid in DCW values decreased substantially as the culture proceeded despite significant presence of glucose into the medium, and concomitant significant rise

in the biosynthesis of endopolysaccharides was observed (in some cases maximum quantities of endopolysaccharides in DCW ≥ 60% w/w were recorded) (Diamantopoulou et al. 2012, 2014, 2016).

Besides nitrogen limitation that is a very important prerequisite in order for lipid production from sugars and related substrates to be carried out, oxygen supply is another critical factor affecting de novo lipid synthesis (Ratledge and Wynn 2002; Ratledge and Cohen 2008). In *M. isabellina* ATHUM 2935 grown in shake flasks, high oxygen uptake was recorded in the balanced growth phase which was significantly decreased during lipid accumulation (Papanikolaou et al. 2004a). In several oleaginous Zygomycetes, agitated flasks may offer substantial oxygen supply; for instance, trials of *M. hiemalis* IRL 51 in highly aerated and agitated

bioreactors (10-L active volume; aeration adjusted to 1 vvm; agitation adjusted to 600 rpm) were compared with the ones carried out in 500-mL baffled flasks, and no statistically significant differences as regards DCW and lipid production as well as GLA quantity (%, w/w) in total lipids were recorded for these two types of cultures (Kennedy et al. 1994). Similarly, not significantly different results as regards DCW and SCO production as well as glucose assimilation were noted between shake-flask and well aerated stirred-tank bioreactor experiments for *M. isabellina* ATHUM 2935 and *T. elegans* CCF-1465 (Chatzifragkou et al. 2010; Zikou et al. 2013) suggesting, thus, that shake-flask experiments for oleaginous Zygomycetes, is an adequate culture mode sufficiently favoring oxygen supply.

Fig. 2 Pathways involved in the intermediate metabolism of glycerol and glucose in order for lipid and polysaccharides to be synthesized. Mitochondrial transport systems: a–c: interlinked puryvate-malate translocase systems; d: citrate-malate translocase. Enzymes: *ACL* ATP-citrate lyase, *FAS* fatty acid synthase enzymatic complex, *ICDH* iso-citrate dehydrogenase, *MD_c* malate dehydrogenase (cytosolic), *MD_m* malate dehydrogenase (mitochondrial), *ME*: NADPH⁺-malic enzyme, *PD* pyruvate dehydrogenase, *CS* citrate synthase, *ICL* iso-citrate lyase, *GK* glycerol kinase, *3-P-GDH* 3-P-glycerol dehydroge-

nase, *HK* hexokinase, *PGI* 6-phosphoglucose isomerase, *PFK* 6-phospho-fructokinase, *ALDO* aldolase A, *GAT* G-3-P acyltransferase, *AGAT* 1-acyl-G-3-P acyltransferase, *PAP* phosphatidic acid phosphohydrolase, *DGA1* and *DGA2* diacylglycerol acyltransferases, *LRO1* phospholipid diacylglycerol acyltransferase [Adapted from Ratledge (1987), Ratledge and Wynn (2002), Papanikolaou et al. (2008), Papanikolaou and Aggelis (2009) and Papanikolaou and Aggelis (2011a)]

The intermediate metabolism of glucose and similarly catabolized compounds (i.e. glycerol) in oleaginous microorganisms is illustrated in Fig. 2.

In the case of de novo lipid synthesis, for the formation of 1 mol of TAG c. 30 mol of $CH_3COSCoA$ are required (Ratledge and Wynn 2002). The stoichiometry of lipid accumulation indicates that in the theoretical case in which all of the $CH_3COSCoA$ produced is channeled towards SCO synthesis, the maximum theoretical yield of SCO produced per glucose consumed is c. 0.32 g/g (Ratledge and Wynn 2002). This value is slightly higher concerning the fermentation of xylose, assuming that xylose-assimilating oleaginous microorganisms utilize exclusively the phospho-ketolase pathway (lipid yield on xylose consumed around 0.34 g/g). If xylose is degraded through the pentose-phosphate pathway this yield is ≈ 0.30 g/g, similar to that obtained on glycerol (Ratledge 1988; Ratledge and Wynn 2002; Papanikolaou and Aggelis 2011a). In practice, the yield obtained by a dedicated oleaginous yeast cultivated in well aerated bioreactor cultures is 22.4 g of oil from 100 g of glucose (Ratledge and Cohen 2008). Thus, it is considered that an almost optimum conversion of 100 g glucose would result in the synthesis of c. 20 g oil plus 30 g oil-free biomass giving a total of 50 g of yeast DCW (Ratledge 1994; Ratledge and Cohen 2008). However, the last years there have been a continuously increasing number of efforts, in order to construct genetically engineered microorganisms (mostly yeasts of the species *Y. lipolytica* and to lesser extent of the species *Rhodosporidium toruloides* and *S. cerevisiae* and bacteria of the species *Escherichia coli*) that would get over this conversion yield threshold of 0.22 g/g. In most of the cases, works centered on the construction of genetically modified strains that: (1) present an increased availability of lipid biosynthetic precursors and mostly glycerol-3-phosphate, through inactivation of the glycerol-3-phosphate dehydrogenase (GUT2) or over-expression of glycerol-3-phosphate dehydrogenase (GPD1 and/or GDP2) (Beopoulos et al. 2008; Dulermo and Nicaud 2011); (2) present an over-expression of enzymes implicated in the lipogenic metabolic pathways like DAG acyltransferases (DGA1 and DGA2) (Beopoulos et al. 2012), malic enzyme (Zhang et al. 2016a) or acetyl-CoA carboxylase (ACC1) (Tai and Stephanopoulos 2013); (3) present a knockout of some crucial enzymes (like 2-methyl-citrate dehydratase), the inactivation of which would mimic nitrogen limitation (Papanikolaou et al. 2013); (4) maximize the cytosolic redox metabolism through conversion of the excess NADH into NADPH, since it had been previously demonstrated that limitation in NADPH impaired lipid synthesis significantly (Qiao et al. 2017); (5) present defective degradation pathways including lipolysis (Dulermo et al. 2013) and β-oxidation process (Mličková et al. 2004; Beopoulos et al. 2008). Indeed, in several cases, genetically modified strains presenting a conversion yield similar or higher than 0.22 g of lipid produced per g of sugar consumed have been constructed.

Table 3 Lipid production by genetically modified yeast and bacterial strains as compared to the results achieved by wild-type *M. isabellina* strain ATHUM 2935 under various cultivation configurations

| Microbial species	Lipid (g/L)	Lipid in DCW (%, w/w)	Conversion yield (g/g)	Substrate	Cultivation mode	References
E. coli[a]	≈ 7	78.0	0.28	Glucose	Batch-bioreactor	Dellomonaco et al. (2011)
E. coli[a]	5.2	n.r.	0.26	Glucose	Batch-bioreactor	Zhang et al. (2012)
S. cerevisiae[b]	2.2	≈ 18	0.12	Glucose	Fed-batch bioreactor	Leber et al. (2015)
R. toruloides[c]	89.4	75.6	0.22	Glucose	Fed-batch bioreactor	Zhang et al. (2016a)
R. toruloides[c]	16.4	61.1	0.23	Glucose	Shake flasks	Zhang et al. (2016b)
Y. lipolytica[d]	28.5	61.7	0.20	Glucose	Batch bioreactor	Tai and Stephanopoulos (2013)
Y. lipolytica[d]	16.1	≈ 88	0.20	Glucose	Batch bioreactor	Blazeck et al. (2014)
Y. lipolytica[d]	55.0	69.0	0.24	Glucose	Fed-batch bioreactor	Qiao et al. (2015)
Y. lipolytica[d]	98.9	66.8	0.27	Glucose	Fed-batch bioreactor	Qiao et al. (2017)
M. isabellina	18.1	50.4	0.18	Glucose	Shake flasks	Papanikolaou et al. (2004b)
M. isabellina	17.6	61.1	0.23	Glucose	Shake flasks	Gardeli et al. (2017)
M. isabellina	8.5	83.3	0.25	Glucose	Shake flasks	Papanikolaou et al. (2017)
M. isabellina	5.4	66.7	0.22	Crude glycerol	Shake flasks	Papanikolaou et al. (2017)

[a]*Escherichia*

[b]*Saccharomyces*

[c]*Rhodosporidium*

[d]*Yarrowia*

Fig. 3 Microscopy of the fungus *M. isabellina* ATHUM 2935 cultivated on glucose (**a**) and biodiesel-derived crude glycerol (**b**). Magnification × 1000. Results of the cultures have appeared in Dourou et al. (2017) and Papanikolaou et al. (2017)
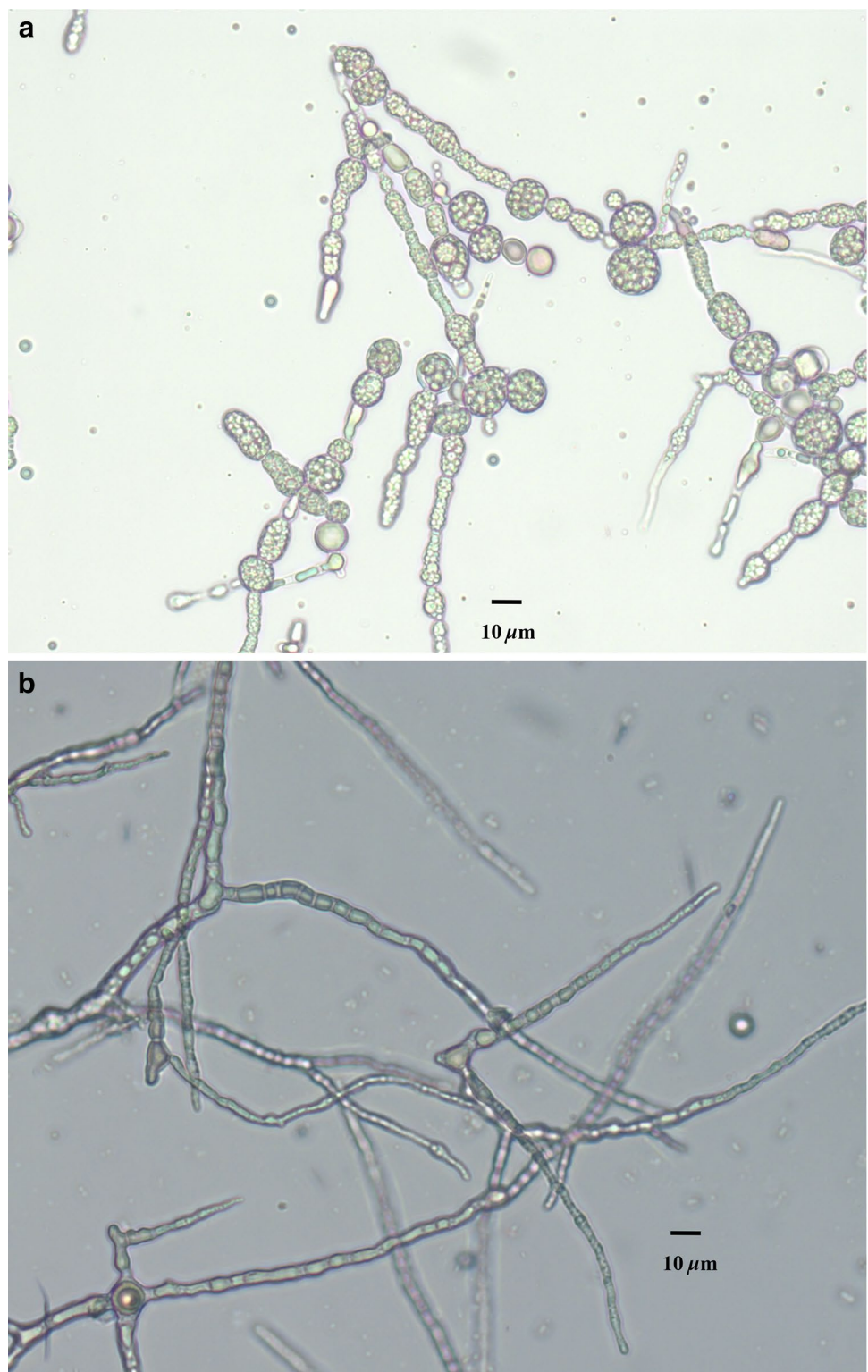
Wild-type strains of *M. isabellina* (mostly the strain ATHUM 2935) have been recorded as some of the very few described in the literature, presenting conversion yield values close or even higher than 0.22 g/g during growth on glucose and/or biodiesel-derived glycerol, comparing favorably to many of the genetically modified strains constructed for this purpose, specifically by taking into consideration the lipid accumulation capacity and the conversion yield of lipid per unit of substrate consumed (Table 3). Growth of *M. isabellina* ATHUM 2935 on glucose was accompanied by the

production of saturated with lipid mycelia whereas numerous substantially lipid-rich chlamydospores also appeared (Dourou et al. 2017), while in contrast growth on glycerol was accompanied only by the production of mycelia containing lipid (in any case, in lower quantities than those on glucose) (Fig. 3a, b). Similar filamentous morphology without presence of lipid-rich chlamydospores has been reported during growth on fructose and molasses. The important presence of oily chlamydospores during growth on glucose may define a rheological behavior of the culture similar to that exhibited by yeasts, favoring potential scale-up of the process which is not evident when glycerol or molasses are used as carbon sources.

Besides *M. isabellina* ATHUM 2935, the various morphological forms of Zygomycetes seem to have an important effect upon the efficiency of SCO production. In the case of another *M. isabellina* strain, it had been demonstrated that distinct morphological forms including pellets of different sizes, free dispersed mycelia, and broken hyphal fragments were developed, while different morphological forms led to variable levels of lipid accumulation as well as to different spatial patterns of lipid distribution within pellets/mycelial aggregates. Specifically, significantly higher lipid content (i.e. 0.75 g of lipid per g of DCW) and lipid yield (0.18 g of lipid per g glucose consumed) were achieved in free dispersed mycelia than in pellets (Gao et al. 2014). On the other hand, the biosynthesis of low-molecular weight extra-cellular metabolites antagonistic to the synthesis of microbial oil (i.e. malic acid) was repressed in free dispersed mycelium form (Gao et al. 2014). Likewise, for the case of strains belonging to the genus *Mucor*, the morphology can significantly be influenced by the culture conditions imposed, and can have important impact upon the synthesis of various metabolic compounds. In detail, *M. genevensis* NRRL 1407 cultivated on low dissolved oxygen tension (DOT) media, was developed under a yeast-like morphology whereas the carbon flow was mostly directed towards the fermentative metabolism and, thus, ethanol instead of lipid production (Rogers et al. 1974). Moreover, in trials performed at carbon- (i.e. glucose or citric acid) excess conditions for *Mucor* sp. strains (i.e. *M. circinelloides* CBS 172-27 and *M. genevensis* NRRL 1407), it has been demonstrated that irrespective of the DOT imposed, fermentation of the carbon source towards ethanol occurred, and thus lower lipid accumulation occurred, whereas metabolic shift was directly correlated with the occurrence of the microorganism under yeast-like morphology (Rogers et al. 1974; Aggelis et al. 1988; Aggelis 1996).

Concerning xylose metabolism, this pentose is initially reduced into xylitol by either NADH- or NADPH-dependent xylose reductase (Sarris and Papanikolaou 2016). Thereafter, xylitol may be secreted from the cell (case of xylitol-producing microorganisms) and/or oxidized to xylulose by a NAD- or NADP-dependent xylitol dehydrogenase, followed by its phosphorylation to yield xylulose-5-P (Zikou et al. 2013; Sarris and Papanikolaou 2016; Gardeli et al. 2017). In the case of *M. isabellina* ATHUM 2935, growth on xylose was accompanied by significantly lower biomass and lipid production compared to that on glucose, since in the former case a non-negligible part of xylose was diverted towards the synthesis of xylitol that was secreted into the growth medium instead of the synthesis of lipid and biomass (i.e. from 80 g/L of initial xylose concentration, xylitol production of *c*. 24 g/L occurred at the end of growth in shake-flask nitrogen-limited trials; see: Gardeli et al. 2017). Remarkable xylitol production, to the detriment of lipid accumulation during growth on xylose, has been also reported for *T. elegans* CCF-1465. This microorganism during its cultivation on xylose employed as the sole substrate, produced *c*. 31 g/L of xylitol about 240 h (10 days) after inoculation, from 100 g/L of xylose, while in trials performed on blends of xylose and glucose, the microorganism produced xylitol quantities that increased with the increment of xylose concentration into the medium (Zikou et al. 2013). Xylitol is a very much appreciated sweetener compound the synthesis of which is carried out by microorganisms included but not limited to yeasts of the genus *Candida* (Ikeuchi et al. 1999) or fungi of the genus *Petromyces* (Dahiya 1991) growing on xylose-based media. Optimization of xylitol production by Zygomycetes like *M. isabellina* and *T. elegans* is likely to be studied in the future.

M. isabellina strains have been cultivated on a plethora of hydrophilic carbon sources as substrates in order for SCO production to be carried out, and in most cases, trials have been carried out in shake flasks, whereas in a restricted number of investigations batch bioreactor experiments have been realized (Chatzifragkou et al. 2010; Kumar et al. 2011; Ruan et al. 2014; Cai et al. 2019). State-of-the-art modeling approaches describing lipid production from various types of sugar-based substrates have also been successfully developed and validated by experimental data (Economou et al. 2011a, b; Meeuwse et al. 2011a, b, 2012). Metrics of lipid production by this species cultivated on several hydrophilic renewable resources are depicted in Table 4. Unfortunately, lipids produced by *M. isabellina* cultivated on hydrophilic carbon sources contain low quantities of the medically and nutritionally important GLA (Table 5). However, due to the abundance of oleic acid, these lipids are perfect precursors for the synthesis of 2nd generation biodiesel (Knothe 2005; Moser 2009), specifically if the precious GLA had previously been recovered (Dyan and Narine 2005). Therefore, wild-type *M. isabellina* strains have already demonstrated excellent performances in relation to the conversion of various types of sugars into microbial oil. Genetic engineering approaches (i.e. over-expression of Δ6 desaturase,

Table 4 Lipid production by oleaginous *M. isabellina* strains growing on hydrophilic carbon sources under various cultivation configurations

Carbon source	Strain	DCW (g/L)	Lipid in DCW (% w/w)	Cultivation mode	References
Glucose (analytical)	CBS 224.35	8.2	20.0	Vortex-bottles	Kendrick and Ratledge (1996)
Glucose (analytical)	ATHUM 2935	13.5	56.3	Shake flasks	Papanikolaou et al. (2004a)
Glucose (analytical)	ATHUM 2935	35.9	50.4	Shake flasks	Papanikolaou et al. (2004b)
Potato starch	ATHUM 2935	10.4	35.6	Shake flasks	Papanikolaou et al. (2007b)
Apple pectin	ATHUM 2935	8.4	23.8	Shake flasks	Papanikolaou et al. (2007b)
Lactose monohydrate	ATHUM 2935	9.5	36.8	Shake flasks	Papanikolaou et al. (2007b)
Glycerol (crude)	ATHUM 2935	8.5	51.7	Shake flasks	Papanikolaou et al. (2008)
Xylose (pure)	ATHUM 2935	8.7	65.5	Shake flasks	Fakas et al. (2009)
Glucose (commercial)	ATHUM 2935	13.2	75.0	Shake flasks	Chatzifragkou et al. (2010)
Fructose (commercial)	ATHUM 2935	12.1	61.2	Shake flasks	Chatzifragkou et al. (2010)
Blackstrap molasses	ATHUM 2935	9.5	53.7	Shake flasks	Chatzifragkou et al. (2010)
Glucose (commercial)	ATHUM 2935	17.8	72.0	Batch bioreactor	Chatzifragkou et al. (2010)
Lactose enriched deproteinized cheese-whey	ATHUM 2935	32.0	25.3	Shake flasks	Vamvakaki et al. (2010)
Sweet sorghum extract	ATHUM 2935	14.8	47.0	Shake flasks	Economou et al. (2011a)
Rice hull hydrolysate	ATHUM 2935	5.6	64.3	Shake flasks	Economou et al. (2011b)
Glucose	KK1	15.9	44.1	Batch bioreactor	Kumar et al. (2011)
Corn stover acid hydrolysate	ATCC 42613	14.1	33.9	Shake flasks	Ruan et al. (2012)
Corn stover alkali hydrolysate	ATCC 42613	10.9	29.5	Shake flasks	Ruan et al. (2012)
Glucose (analytical)	ATCC 42613	22.9	44.5	Shake flasks	Ruan et al. (2012)
Xylose (analytical)	ATCC 42613	21.6	40.7	Shake flasks	Ruan et al. (2012)
Corn fiber hydrolysate	M2	18.2	45.7	Shake flasks	Xing et al. (2012)
Deproteinized cheese-whey	DSM 1414	11.5	31.0	Shake flasks	Demir et al. (2013)
Deproteinized cheese-whey previously treated with lactase	DSM 1414	26.6	64.5	Shake flasks	Demir et al. (2013)
Corn stover hydrolysate	ATCC 42613	12.8	24.8	Shake flasks	Ruan et al. (2013)
Giant reed hydrolysate	ATCC 42613	13.8	21.2	Shake flasks	Ruan et al. (2013)
Corn stover hydrolysate (1:1 w/w acid- and alkali- hydrolysate)	ATCC 42613	17.2	29.7	Shake flasks	Ruan et al. (2014)
Corn stover hydrolysate (1:1 w/w acid- and alkali- hydrolysate)	ATCC 42613	18.7	37.0	Batch bioreactor	Ruan et al. (2014)
Synthetic hydrolysate that mimics corn stover hydrolysate	ATCC 42613	14.8	36.6	Shake flasks	Ruan et al. (2014)
Blends of glucose, xylose and acetate (control)	ATCC 42613	9.3	43.3	Shake flasks	Ruan et al. (2015)
Blends of glucose, xylose and acetate and inhibitors (HMF, furfural, cumaric acid, etc)	ATCC 42613	6.7	38.0	Shake flasks	Ruan et al. (2015)
Steam exploded corn stover (various enzymatic hydrolysates)	Isolate not indicated	16.8	55.8	Shake flasks	Fang et al. (2016)
Steam exploded corn stover (various enzymatic hydrolysates)	Isolate not indicated	15.0	57.3	Shake flasks	Fang et al. (2016)
Steam exploded corn stover (various enzymatic hydrolysates)	Isolate not indicated	36.1	51.8	Shake flasks	Fang et al. (2016)
Glucose and xylose (50–50% w/w)	NRRL 1757	18.8	50.5	Shake flasks	Harde et al. (2016)
Xylose (analytical)	NRRL 1757	15.3	66.0	Shake flasks	Harde et al. (2016)
Glucose (analytical)	NRRL 1757	20.0	47.5	Shake flasks	Harde et al. (2016)
Glucose (commercial)	ATHUM 2935	28.8	61.0	Shake flasks	Gardeli et al. (2017)
Xylose (commercial)	ATHUM 2935	20.7	37.2	Shake flasks	Gardeli et al. (2017)
Glucose/xylose blend	ATHUM 2935	26.1	59.7	Shake flasks	Gardeli et al. (2017)

Table 4 (continued)

Carbon source	Strain	DCW (g/L)	Lipid in DCW (% w/w)	Cultivation mode	References
Corn stover hydrolysate (sodium hydroxide pretreatment)	Isolate not indicated	23.5	58.3	Shake flasks	Zhao et al. (2017)
Corn stover hydrolysate (aqueous ammonia pretreatment)	Isolate not indicated	14.4	52.1	Shake flasks	Zhao et al. (2017)
Corn stover hydrolysate (lime pretreatment)	Isolate not indicated	13.9	56.1	Shake flasks	Zhao et al. (2017)
Corn stover hydrolysate (steam explosion pretreatment)	Isolate not indicated	15.7	56.7	Shake flasks	Zhao et al. (2017)
Glucose (commercial)	ATHUM 2935	10.2	83.3	Shake flasks	Papanikolaou et al. (2017)
Glycerol (crude)	ATHUM 2935	8.1	66.7	Shake flasks	Papanikolaou et al. (2017)
Sodium hydroxide pretreated rice straw (various enzymatic hydrolysates)	Isolate not indicated	15.2	53.3	Shake flasks	Zhao et al. (2019)
Sodium hydroxide pretreated rice straw (various enzymatic hydrolysates)	Isolate not indicated	28.3	51.9	Shake flasks	Zhao et al. (2019)
Sodium hydroxide pretreated rice straw (various enzymatic hydrolysates)	Isolate not indicated	14.0	55.7	Shake flasks	Zhao et al. (2019)
Glycerol-enriched sugarcane bagasse hydrolysate	NRRL 1757	7.2	52.3	Shake flasks	Cai et al. (2019)
Glycerol-enriched sugarcane bagasse hydrolysate	NRRL 1757	19.6	64.0	Batch bioreactor	Cai et al. (2019)

Table 5 Fatty acid composition (in %, w/w) of *M. isabellina* ATHUM 2935 total cellular lipids obtained during growth on several types of hydrophilic carbon sources under nitrogen-limited conditions

	C16:0	C18:0	$^{\Delta 9}$C18:1	$^{\Delta 9,12}$C18:2	$^{\Delta 6,9,12}$C18:3	References
Glucose	19.8	2.6	53.2	15.0	3.4	Gardeli et al. (2017)
Xylose	24.5	13.7	37.3	13.6	5.1	Gardeli et al. (2017)
Crude glycerol	22.0	3.9	51.2	18.8	4.0	Papanikolaou et al. (2017)
Fructose	28.5	2.2	51.3	9.6	2.5	Chatzifragkou et al. (2010)
Molasses	23.1	2.4	51.4	15.2	3.5	Chatzifragkou et al. (2010)
Cheese-whey	24.5	4.2	49.1	14.3	3.8	Vamvakaki et al. (2010)
Crude glycerol	21.2	5.2	52.0	13.5	3.8	Papanikolaou et al. (2008)
Lactose	23.0	1.9	56.5	11.0	4.1	Papanikolaou et al. (2007b)
Starch	26.5	2.1	56.5	10.5	4.0	Papanikolaou et al. (2007b)
Pectin	22.8	6.8	42.5	14.5	6.1	Papanikolaou et al. (2007b)

Fatty acid composition presented at the stationary growth phase. In all cases lipid in DCW values were higher than 25% w/w

over-expression of FA elongases, etc) could be applied and could potentially drastically improve the FA composition (viz. the concentration of GLA or other long-chain PUFAs) in these cellular lipids.

Concluding remarks

SCOs present several potential applications since they can be implicated as precursors for the synthesis of lipid-based biofuels (biodiesel or renewable diesel) and can be employed as substitutes of expensive lipids rarely found in the plant or animal kingdom. Various Zygomycetes species and specifically these of the genus *Mortierella* (with the exception of some strains of the species *M. wolfii*—Davies and Wobeser 2010) are non-pathogenic. Due to their potentiality to grow on several types of waste- and residue-type substrates these microorganisms are considered as perfect microbial cell factories for the production of SCOs. Fatty materials have been employed as substrates (or co-substrates) by oleaginous Zygomycetes, and in most cases the extra-cellular addition of fat substrate

remarkably enhanced lipid accumulation inside the fungal mycelia. In several cases, biomodification of the employed fatty materials occurred, and cellular lipids presented a FA composition that did not previously exist. Moreover, some wild-type strains of *M. isabellina* cultivated on glucose, have presented exceptional lipid production. Conversion yields of lipid produced per unit of glucose consumed reported, were in several cases comparable to the highest values achieved for genetically engineered SCO-producing yeast or bacterial strains. Therefore, production of lipid by *M. isabellina* strains is a promising bioprocess which may be appreciated by the chemical industry in the near future.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

References

Aggelis G (1996) Two alternative pathways for substrate assimilation by *Mucor circinelloides*. Folia Microbiol 41:254–256

Aggelis G, Ratomahenina R, Arnaud A, Galzy P, Martin-Privat P, Perraud JP, Pina M, Graille J (1988) Etude de l'influence des conditions de culture sur la teneur en acide gamma linolénique de souches de *Mucor*. Oléagineux 43:311–317

Aggelis G, Komaitis M, Papanikolaou S, Papadopoulos G (1995a) A mathematical model for the study of lipid accumulation in oleaginous microorganisms. I. Lipid accumulation during growth of *Mucor circinelloides* CBS 172-27 on a vegetable oil. Gracas y Aceites 46:169–173

Aggelis G, Komaitis M, Papanikolaou S, Papadopoulos G (1995b) A mathematical model for the study of lipid accumulation in oleaginous micro-organisms: II. Study of cellular lipids of *Mucor circinelloides* CBS 172-27 during growth on a vegetable oil. Grasas Aceites 46:245–250

Aggelis G, Papadiotis G, Komaitis M (1997) Microbial fatty acid specificity. Folia Microbiol 42:117–120

Alakhras R, Bellou S, Fotaki G, Stephanou G, Demopoulos N, Papanikolaou S, Aggelis G (2015) Fatty acid lithium salts from *Cunninghamella echinulata* have cytotoxic and genotoxic effects on HL-60 human leukemia cells. Eng Life Sci 15:243–253

Aoki H, Miyamoto N, Furuya Y, Mankura M, Endo Y, Fujimoto K (2002) Incorporation and accumulation of docosahexaenoic acid from the medium by *Pichia methanolica* HA-32. Biosci Biotechnol Biochem 66:2632–2638

Athenaki M, Gardeli C, Diamantopoulou P, Tchakouteu SS, Sarris D, Philippoussis A, Papanikolaou S (2018) Lipids from yeasts and fungi: physiology, production and analytical considerations. J Appl Microbiol 124:336–367

Barre E (2009) Borage, evening primrose, blackcurrant, and fungal oils: γ-linolenic acid-rich oils. In: Moreau RA, Kamal-Eldin A (eds) Gourmet and health-promoting specialty oils. Academic Press and AOCS Press, Champaign, pp 237–266

Bati N, Hammond EG, Glatz BA (1984) Biomodification of fats and oils: trials with *Candida lipolytica*. J Am Oil Chem Soc 61:1743–1746

Bellou S, Aggelis G (2012) Biochemical activities in *Chlorella* sp. and *Nannochloropsis salina* during lipid and sugar synthesis in a lab-scale open pond simulating reactor. J Biotechnol 164:318–329

Bellou S, Moustogianni A, Makri A, Aggelis G (2012) Lipids containing polyunsaturated fatty acids synthesized by zygomycetes grown on glycerol. Appl Biochem Biotechnol 166:146–158

Bellou S, Baeshen MN, Elazzazy AM, Aggeli D, Sayegh F, Aggelis G (2014) Microalgal lipids biochemistry and biotechnological perspectives. Biotechnol Adv 32:1476–1493

Bellou S, Triantaphyllidou IE, Aggeli D, Elazzazy AM, Baeshen MN, Aggelis G (2016) Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. Curr Opin Biotechnol 37:24–35

Beopoulos A, Mrozova Z, Thevenieau F, Le Dall MT, Hapala I, Papanikolaou S, Chardot T, Nicaud JM (2008) Control of lipid accumulation in the yeast *Yarrowia lipolytica*. Appl Environ Microbiol 74:7779–7789

Beopoulos A, Haddouche R, Kabran P, Dulermo T, Chardot T, Nicaud JM (2012) Identification and characterization of DGA2, an acyltransferase of the DGAT1 acyl-CoA:diacylglycerol acyltransferase family in the oleaginous yeast *Yarrowia lipolytica*. New insights into the storage lipid metabolism of oleaginous yeasts. Appl Microbiol Biotechnol 93:1523–1537

Blazeck J, Hill A, Liu L, Knight R, Miller J, Pan A, Otoupal P, Alper HS (2014) Harnessing *Yarrowia lipolytica* lipogenesis to create a platform for lipid and biofuel production. Nat Commun 5:3131

Cai G, Moghaddam L, O'Hara IM, Zhang Z (2019) Microbial oil production from acidified glycerol pretreated sugarcane bagasse by *Mortierella isabellina*. RSC Adv 9:2539–2550

Carsanba E, Papanikolaou S, Erten H (2018) Production of oils and fats by oleaginous microorganisms with an emphasis given to the potential of the nonconventional yeast *Yarrowia lipolytica*. Crit Rev Biotechnol 38:1230–1243

Čertik M, Shimizu S (1999) Biosynthesis and regulation of microbial polyunsaturated fatty acid production. J Biosci Bioeng 87:1–14

Čertik M, Balteszova L, Sajbidor J (1997) Lipid formation and γ-linolenic acid production by Mucorales fungi grown on sunflower oil. Lett Appl Microbiol 25:101–105

Chatzifragkou A, Fakas S, Galiotou-Panayotou M, Komaitis M, Aggelis G, Papanikolaou S (2010) Commercial sugars as substrates for lipid accumulation in *Cunninghamella echinulata* and *Mortierella isabellina*. Eur J Lipid Sci Technol 112:1048–1057

Dahiya JS (1991) Xylitol production by *Petromyces albertensis* grown on medium containing D-xylose. Can J Microbiol 37:14–18

Daskalaki A, Vasiliadou IA, Bellou S, Tomaszewska-Hetman L, Chatzikotoula C, Kompoti B, Papanikolaou S, Vayenas D, Pavlou S, Aggelis G (2018) Data on cellular lipids of *Yarrowia lipolytica* grown on fatty substrates. Data Brief 21:1037–1044

Davies RJ (1988) Yeast oil from cheese whey—process development. In: Moreton RS (ed) Single cell oil. Longman Scientific & Technical, Longman House, Burnt Mill, Harlow, pp 99–146

Davies RJ, Holdsworth JE (1992) Synthesis of lipids in yeasts: biochemistry, physiology and production. Adv Appl Lipid Res 1:119–159

Davies JL, Wobeser GA (2010) Systemic infection with *Mortierella wolfii* following abortion in a cow. Can Vet J 51:1391–1393

Dellomonaco C, Clomburg JM, Miller EN, Gonzalez R (2011) Engineered reversal of the β-oxidation cycle for the synthesis of fuels and chemicals. Nature 476:355–359

Demir M, Turhan I, Kucukcetin A, Alpkent Z (2013) Oil production by *Mortierella isabellina* from whey treated with lactase. Bioresour Technol 128:365–369

Diamantopoulou P, Papanikolaou S, Katsarou E, Komaitis M, Aggelis G, Philippoussis A (2012) Mushroom polysaccharides and lipids synthesized in liquid agitated and static cultures. Part II: study of *Volvariella volvacea*. Appl Biochem Biotechnol 167:1890–1906

Diamantopoulou P, Papanikolaou S, Komaitis M, Aggelis G, Philippoussis A (2014) Patterns of major metabolites biosynthesis by

different mushroom fungi grown on glucose-based submerged cultures. Bioprocess Biosyst Eng 37:1385–1400

Diamantopoulou P, Papanikolaou S, Aggelis G, Philippoussis A (2016) Adaptation of *Volvariella volvacea* metabolism in high carbon to nitrogen ratio media. Food Chem 196:272–280

Diwan B, Parkhey P, Gupta P (2018) From agro-industrial wastes to single cell oils: a step towards prospective biorefinery. Folia Microbiol 63:547–568

Dourou M, Mizerakis P, Papanikolaou S, Aggelis G (2017) Storage lipid and polysaccharide metabolism in *Yarrowia lipolytica* and *Umbelopsis isabellina*. Appl Microbiol Biotechnol 101:7213–7226

Dourou M, Aggeli D, Papanikolaou S, Aggelis G (2018) Critical steps in carbon metabolism affecting lipid accumulation and their regulation in oleaginous microorganisms. Appl Microbiol Biotechnol 102:2509–2523

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

Dulermo T, Tréton B, Beopoulos A, Kabran Gnankon AP, Haddouche R, Nicaud JM (2013) Characterization of the two intracellular lipases of *Y. lipolytica* encoded by TGL3 and TGL4 genes: new insights into the role of intracellular lipases and lipid body organisation. Biochim Biophys Acta 1831:1486–1495

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

Economou CN, Aggelis G, Pavlou S, Vayenas DV (2011a) Modelling of single-cell oil production under nitrogen limited and substrate inhibition conditions. Biotechnol Bioeng 108:1049–1055

Economou CN, Aggelis G, Pavlou S, Vayenas DV (2011b) Single cell oil production from rice hulls hydrolysate. Bioresour Technol 102:9737–9742

Fakas S, Papanikolaou S, Batsos A, Galiotou-Panayotou M, Mallouchos 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

Fang H, Zhao C, Chen S (2016) Single cell oil production by *Mortierella isabellina* from steam exploded corn stover degraded by three-stage enzymatic hydrolysis in the context of on-site enzyme production. Bioresour Technol 216:988–995

Fickers P, Benetti PH, Waché Y, Marty A, Mauersberger S, Smit MS, Nicaud JM (2005) Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. FEMS Yeast Res 5:527–543

Gao D, Zeng J, Yu X, Dong T, Chen S (2014) Improved lipid accumulation by morphology engineering of oleaginous fungus *Mortierella isabellina*. Biotechnol Bioeng 111:1758–1766

Gardeli C, Athenaki M, Xenopoulos E, Mallouchos A, Koutinas AA, Aggelis G, Papanikolaou S (2017) Lipid production and characterization by *Mortierella* (*Umbelopsis*) *isabellina* cultivated on lignocellulosic sugars. J Appl Microbiol 123:1461–1477

Harde SM, Wang Z, Horne M, Zhu JY, Pan X (2016) Microbial lipid production from SPORL-pretreated Douglas fir by *Mortierella isabellina*. Fuel 175:64–74

Huang C, Chen XF, Xiong L, Chen XD, Ma LL, Chen Y (2013) Single cell oil production from low-cost substrates: the possibility and potential of its industrialization. Biotechnol Adv 31:129–139

Ikeuchi T, Azuma M, Kato J, Ooshima H (1999) Screening of microorganisms for xylitol production and fermentation behavior in high concentrations of xylose. Biomass Bioenergy 16:333–339

Kendrick A, Ratledge C (1996) Cessation of polyunsaturated fatty acid formation in four selected filamentous fungi when grown on plant oils. J Am Oil Chem Soc 73:431–435

Kennedy MJ, Reader SL, Davies J, Rhoades DA, Silby HW (1994) The scale up of mycelial shake flask fermentations: a case study of gamma linolenic acid production by *Mucor hiemalis* IRL 51. J Ind Microbiol 13:212–216

Knothe G (2005) Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process Technol 86:1059–1070

Koritala S, Hesseltine CW, Pryde EH, Mounts TL (1987) Biochemical modification of fats by microorganisms: a preliminary study. J Am Oil Chem Soc 64:509–513

Koutinas AA, Chatzifragkou A, Kopsahelis N, Papanikolaou S, Kookos IK (2014a) Design and techno-economic evaluation of microbial oil production as a renewable resource for biodiesel and oleochemical production. Fuel 116:566–577

Koutinas AA, Vlysidis A, Pleissner D, Kopsahelis N, Garcia IL, Kookos IK, Papanikolaou S, Kwan TH, Lin SKC (2014b) Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. Chem Soc Rev 43:2587–2627

Kumar I, Ramalakshmi MA, Sivakumar U, Santhanakrishnan P, Zhan X (2011) Production of microbial oils from *Mortierella* sp. for generation of biodiesel livestock. Afr J Microbiol Res 5:4105–4111

Leber C, Polson B, Fernandez-Moya R, Da Silva NA (2015) Overproduction and secretion of free fatty acids through disrupted neutral lipid recycle in *Saccharomyces cerevisiae*. Metab Eng 28:54–62

Matsuo T, Terashima M, Hashimoto Y, Hasida W (1981) Method for producing cacao butter substitute. US Patent 4,308,350

Meeuwse P, Tramper J, Rinzema A (2011a) Modeling lipid accumulation in oleaginous fungi in chemostat cultures: I. Development and validation of a chemostat model for *Umbelopsis isabellina*. Bioprocess Biosyst Eng 34:939–949

Meeuwse P, Tramper J, Rinzema A (2011b) Modeling lipid accumulation in oleaginous fungi in chemostat cultures. II: validation of the chemostat model using yeast culture data from literature. Bioprocess Biosyst Eng 34:951–961

Meeuwse P, Akbari P, Tramper J, Rinzema A (2012) Modeling growth, lipid accumulation and lipid turnover in submerged batch cultures of *Umbelopsis isabellina*. Bioprocess Biosyst Eng 35:591–603

Meeuwse P, Sanders JPM, Tramper J, Rinzema A (2013) Lipids from yeasts and fungi: tomorrow's source of biodiesel? Biofuel Bioprod Biorefin 7:512–524

Metzger JO, Bornscheuer U (2006) Lipids as renewable resources: current state of chemical and biotechnological conversion and diversification. Appl Microbiol Biotechnol 71:13–22

Meyer KH, Schweizer E (1976) Control of fatty-acid synthetase levels by exogenous long-chain fatty acids in the yeasts *Candida lipolytica* and *Saccharomyces cerevisiae*. Eur J Biochem 65:317–324

Mličková K, Luo Y, D'Andrea S, Peč P, Chardot T, Nicaud JM (2004) Acyl-CoA oxidase, a key step for lipid accumulation in the yeast *Yarrowia lipolytica*. J Mol Catal B 28:81–85

Moser BR (2009) Biodiesel production, properties, and feedstocks. In Vitro Cell Develop Biol Plant 45:229–266

Papanikolaou S, Aggelis G (2009) Biotechnological valorization of biodiesel derived glycerol waste through production of single cell oil and citric acid by *Yarrowia lipolytica*. Lipid Technol 21:83–87

Papanikolaou S, Aggelis G (2010) *Yarrowia lipolytica*: a model microorganism used for the production of tailor-made lipids. Eur J Lipid Sci Technol 112:639–654

Papanikolaou S, Aggelis G (2011a) Lipids of oleaginous yeasts. Part I: biochemistry of single cell oil production. Eur J Lipid Sci Technol 113:1031–1051

Papanikolaou S, Aggelis G (2011b) Lipids of oleaginous yeasts. Part II: technology and potential applications. Eur J Lipid Sci Technol 113:1052–1073

Papanikolaou S, Chevalot I, Komaitis M, Aggelis G, Marc I (2001) Kinetic profile of the cellular lipid composition in an oleaginous *Yarrowia lipolytica* capable of producing a cocoa-butter substitute from industrial fats. Antonie Van Leeuwenhoek 80:215–224

Papanikolaou S, Chevalot I, Komaitis M, Marc I, Aggelis G (2002) Single cell oil production by *Yarrowia lipolytica* growing on an industrial derivative of animal fat in batch cultures. Appl Microbiol Biotechnol 58:308–312

Papanikolaou S, Muniglia L, Chevalot I, Aggelis G, Marc I (2003) Accumulation of a cocoa-butter-like lipid by *Yarrowia lipolytica* cultivated on agro-industrial residues. Curr Microbiol 46:124–130

Papanikolaou S, Sarantou S, Komaitis M, Aggelis G (2004a) Repression of reserve lipid turnover in *Cunninghamella echinulata* and *Mortierella isabellina* cultivated in multiple-limited media. J Appl Microbiol 97:867–875

Papanikolaou S, Komaitis M, Aggelis G (2004b) Single cell oil (SCO) production by *Mortierella isabellina* grown on high-sugar content media. Bioresour Technol 95:287–291

Papanikolaou S, Galiotou-Panayotou M, Chevalot I, Komaitis M, Marc I, Aggelis G (2006) Influence of glucose and saturated free-fatty acid mixtures on citric acid and lipid production by *Yarrowia lipolytica*. Curr Microbiol 52:134–142

Papanikolaou S, Chevalot I, Galiotou-Panayotou M, Komaitis M, Marc I, Aggelis G (2007a) Industrial derivative of tallow: a promising renewable substrate for microbial lipid, single-cell protein and lipase production by *Yarrowia lipolytica*.. Electron J Biotechnol 10:425–435

Papanikolaou S, Galiotou-Panayotou M, Fakas S, Komaitis M, Aggelis G (2007b) Lipid production by oleaginous Mucorales cultivated on renewable carbon sources. Eur J Lipid Sci Technol 109:1060–1070

Papanikolaou S, Fakas S, Fick M, Chevalot I, Galiotou-Panayotou M, Komaitis M, Marc I, Aggelis G (2008) Biotechnological valorisation of raw glycerol discharged after bio-diesel (fatty acid methyl-esters) manufacturing process: production of 1,3-propanediol, citric acid and single cell oil. Biomass Bioenergy 32:60–71

Papanikolaou S, Dimou A, Fakas S, Diamantopoulou P, Philippoussis A, Galiotou-Panayotou M, Aggelis G (2011) Biotechnological conversion of waste cooking olive oil into lipid-rich biomass using *Aspergillus* and *Penicillium* strains. J Appl Microbiol 110:1138–1150

Papanikolaou S, Beopoulos A, Koletti A, Thevenieau F, Koutinas AA, Nicaud JM, Aggelis G (2013) Importance of the methyl-citrate cycle on glycerol metabolism in the yeast *Yarrowia lipolytica*. J Biotechnol 168:303–314

Papanikolaou S, Rontou M, Belka A, Athenaki M, Gardeli C, Mallouchos A, Kalantzi O, Koutinas AA, Kookos IK, Zeng AP, Aggelis G (2017) Conversion of biodiesel-derived glycerol into biotechnological products of industrial significance by yeast and fungal strains. Eng Life Sci 17:262–281

Patel A, Arora N, Sartaj K, Pruthi V, Pruthi PA (2016) Sustainable biodiesel production from oleaginous yeasts utilizing hydrolysates of various non-edible lignocellulosic biomasses. Renew Sustain Energy Rev 62:836–855

Patel A, Arora N, Mehtani J, Pruthi V, Pruthi PA (2017) Assessment of fuel properties on the basis of fatty acid profiles of oleaginous yeast for potential biodiesel production. Renew Sustain Energy Rev 77:604–616

Philippoussis A, Diamantopoulou P (2012) Exploitation of the biotechnological potential of agro-industrial by-products through mushroom cultivation. In: Petre M, Berovic M (eds) Mushroom biotechnology and bioengineering. University of Pitesti, CD Press, Bucharest, pp 161–184

Qiao K, Imam Abidi SH, Liu H, Zhang H, Chakraborty S, Watson N, Kumaran Ajikumar P, Stephanopoulos G (2015) Engineering lipid overproduction in the oleaginous yeast *Yarrowia lipolytica*. Metab Eng 29:56–65

Qiao K, Wasylenko TM, Zhou K, Xu P, Stephanopoulos G (2017) Lipid production in *Yarrowia lipolytica* is maximized by engineering cytosolic redox metabolism. Nat Biotechnol 35:173–177

Qin L, Liu L, Zeng AP, Wei D (2017) From low-cost substrates to single cell oils synthesized by oleaginous yeasts. Bioresour Technol 245:1507–1519

Ratledge C (1987) Lipid biotechnology: a wonderland for the microbial physiologist. J Am Oil Chem Soc 64:1647–1656

Ratledge C (1988) Biochemistry, stoichiometry, substrates and economics. In: Moreton RS (ed) Single cell oil. Longman Scientific & Technical, Longman House, Burnt Mill, Harlow, pp 33–70

Ratledge C (1994) Yeasts, moulds, algae and bacteria as sources of lipids. In: Kamel BS, Kakuda Y (eds) Technological advances in improved and alternative sources of lipids. Blackie Academic and Professional, London, pp 235–291

Ratledge C (2011) Are algal oils realistic options for biofuels? Eur J Lipid Sci Technol 113:135–136

Ratledge C (2013a) Microbial oils: an introductory overview of current status and future prospects. Oilseeds Fats Crops Lipids (OCL) 20:D602

Ratledge C (2013b) Oils from microalgae: achievements and prospects http://bioprosp.com/programme/2013/oils-microalgae-achievements-and-prospects. Accessed 22 Mar 2017

Ratledge C, Cohen Z (2008) Microbial and algal oils: do they have a future for biodiesel or as commodity oils? Lipid Technol 20:155–160

Ratledge C, Wynn JP (2002) The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Adv Appl Microbiol 51:1–51

Rogers PJ, Clark-Walker GD, Stewart PR (1974) Effects of oxygen and glucose on energy metabolism and dimorphism of *Mucor genevensis* grown in continuous culture: reversibility of yeast-mycelium conversion. J Bacteriol 119:282–293

Roux-Van der Merwe MP, Badenhorst J, Britz TJ (2005) Fungal treatment of an edible-oil-containing industrial effluent. World J Microbiol Biotechnol 21:947–953

Ruan Z, Zanotti M, Wang X, Ducey C, Liu Y (2012) Evaluation of lipid accumulation from lignocellulosic sugars by *Mortierella isabellina* for biodiesel production. Bioresour Technol 110:198–205

Ruan Z, Zanotti M, Zhong Y, Liao W, Ducey C, Liu Y (2013) Co-hydrolysis of lignocellulosic biomass for microbial lipid accumulation. Biotechnol Bioeng 110:1039–1049

Ruan Z, Zanotti M, Archer S, Liao W, Liu Y (2014) Oleaginous fungal lipid fermentation on combined acid- and alkali-pretreated corn stover hydrolysate for advanced biofuel production. Bioresour Technol 163:12–17

Ruan Z, Hollinshead W, Isaguirre C, Tang YJ, Liao W, Liu Y (2015) Effects of inhibitory compounds in lignocellulosic hydrolysates on *Mortierella isabellina* growth and carbon utilization. Bioresour Technol 183:18–24

Rywińska A, Juszczyk P, Wojtatowicz M, Robak M, Lazar Z, Tomaszewska L, Rymowicz W (2013) Glycerol as a promising substrate for *Yarrowia lipolytica* biotechnological applications. Biomass Bioenergy 48:148–166

Sakuradani E, Shimizu S (2009) Single cell oil production by *Mortierella alpina*. J Biotechnol 114:31–36

Sarris D, Papanikolaou S (2016) Biotechnological production of ethanol: biochemistry, processes and technologies. Eng Life Sci 16:307–329

Sayegh F, Elazzazy A, Bellou S, Moustogianni A, Elkady AI, Baeshen MN, Aggelis G (2016) Production of polyunsaturated

single cell oils possessing antimicrobial and anticancer properties. Ann Microbiol 66:937–948

Saygün A, Şahin-Yeşilçubuk N, Aran N (2014) Effects of different oil sources and residues on biomass and metabolite production by *Yarrowia lipolytica* YB 423–12. J Am Oil Chem Soc 91:1521–1530

Szczęsna-Antczak M, Kubiak A, Antczak T, Bielecki S (2006a) Enzymatic biodiesel synthesis—key factors affecting efficiency of the process. Renew Energy 34:1185–1194

Szczęsna-Antczak M, Antczak T, Piotrowicz-Wasiak M, Rzyska M, Binkowska N, Bielecki S (2006b) Relationships between lipases and lipids in mycelia of two *Mucor* strains. Enzyme Microb Technol 39:1214–1222

Szczęsna-Antczak M, Struszczyk-Świta K, Rzyska M, Szeląg J, Stańczyk Ł, Antczak T (2018) Oil accumulation and *in situ* trans/esterification by lipolytic fungal biomass. Bioresour Technol 265:110–118

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

Takeda I, Tamano K, Yamane N, Ishii T, Miura A, Umemura M, Terai G, Baker SE, Koike H, Machida M (2014) Genome sequence of the Mucoromycotina fungus *Umbelopsis isabellina*, an effective producer of lipids. Genome Announc 2:e00071–e00014

Tatsumi C, Hashimoto Y, Terashima M, Matsuo T (1977) Method for producing cacao butter substitute. US Patent 4,032,405

Tauk-Tornisielo SM, Arasato LS, de Almeida AF, Govone JS, Malagutti EN (2009) Lipid formation and γ-linolenic acid production by *Mucor circinelloides* and *Rhizopus* sp., grown on vegetable oil. Braz J Microbiol 40:342–345

Tchakouteu S, Chatzifragkou A, Kalantzi O, Koutinas AA, Aggelis G, Papanikolaou S (2015) Oleaginous yeast *Cryptococcus curvatus* exhibits interplay between biosynthesis of intracellular sugars and lipids. Eur J Lipid Sci Technol 117:657–672

Tzirita M, Papanikolaou S, Chatzifragkou A, Quilty B (2018) Waste fat biodegradation and biomodification by *Yarrowia lipolytica* and a bacterial consortium composed of *Bacillus* spp. and *Pseudomonas putida*. Eng Life Sci 18:932–942

Vamvakaki AN, Kandarakis I, Kaminarides S, Komaitis M, Papanikolaou S (2010) Cheese whey as a renewable substrate for microbial lipid and biomass production by zygomycetes. Eng Life Sci 10:348–360

Vasiliadou IA, Bellou S, Daskalaki A, Tomaszewska-Hetman L, Chatzikotoula C, Kompoti B, Papanikolaou S, Vayenas D, Pavlou S, Aggelis G (2018) Biomodification of fats and oils and scenarios of adding value on renewable fatty materials through microbial fermentations: modelling and trials with *Yarrowia lipolytica*. J Clean Prod 200:1111–1129

Wagner L, Stielow B, Hoffmann K, Petkovits T, Papp T, Vágvölgyi C, de Hoog GS, Verkley G, Voigt K (2013) A comprehensive molecular phylogeny of the *Mortierellales* (*Mortierellomycotina*) based on nuclear ribosomal DNA. Persoonia 30:77–93

Weber RWS, Tribe HT (2003) Oil as a substrate for *Mortierella* species. Mycologist 17:134–139

Woodbine M (1959) Microbial fat: microorganisms as potential fat producers. Prog Ind Microbiol 1:181–238

Wynn JP, Ratledge C (2005) Microbial production of oils and fats. In: Shetty K, Paliyath G, Pometto A, Levin RE (eds) Food Biotechnology, 2nd edn. CRC Press, Boca Raton FL, pp 443–472

Xing D, Wang H, Pan A, Wang J, Xue D (2012) Assimilation of corn fiber hydrolysates and lipid accumulation by *Mortierella isabellina*. Biomass Bioenergy 39:494–501

Xiong D, Zhang H, Xie Y, Tang N, Berenjian A, Song Y (2015) Conversion of mutton fat to cocoa butter equivalent by increasing the unsaturated fatty acids at the sn-2 position of triacylglycerol through fermentation by *Yarrowia lipolytica*. Am J Biochem Biotechnol 11:57–65

Zhang F, Ouellet M, Batth TS, Adams PD, Petzold CJ, Mukhopadhyay A, Keasling JD (2012) Enhancing fatty acid production by the expression of the regulatory transcription factor FadR. Metab Eng 14:653–660

Zhang S, Ito M, Skerker JM, Arkin AP, Rao CV (2016a) Metabolic engineering of the oleaginous yeast *Rhodosporidium toruloides* IFO0880 for lipid overproduction during high-density fermentation. Appl Microbiol Biotechnol 100:9393–9405

Zhang S, Skerker JM, Rutter CD, Maurer MJ, Arkin AP, Rao CV (2016b) Engineering *Rhodosporidium toruloides* for increased lipid production. Biotechnol Bioeng 113:1056–1066

Zhao C, Deng L, Fang H, Chen S (2017) Microbial oil production by *Mortierella isabellina* from corn stover under different pretreatments. RSC Adv 7:56239–56246

Zhao C, Xie B, Zhao R, Fang H (2019) Microbial oil production by *Mortierella isabellina* from sodium hydroxide pretreated rice straw degraded by three-stage enzymatic hydrolysis in the context of on-site cellulase production. Renew Energy 130:281–289

Zhong JJ, Tang YJ (2004) Submerged cultivation of medicinal mushrooms for production of valuable bioactive metabolites. Adv Biochem Eng Biotechnol 87:25–59

Zikou E, Chatzifragkou A, Koutinas AA, Papanikolaou S (2013) Evaluating glucose and xylose as cosubstrates for lipid accumulation and γ-linolenic acid biosynthesis of *Thamnidium elegans*. J Appl Microbiol 114:1020–1032