Published in final edited form as:
Bioresour Technol. 2013 September ; 144: . doi:10.1016/j.biortech.2013.06.047.

Manipulation of culture conditions alters lipid content and fatty acid profiles of a wide variety of known and new oleaginous yeasts species

Irnayuli R. Sitepu[a,b,*], **Ryan Sestric**[c], **Laura Ignatia**[a], **David Levin**[c], **J. Bruce German**[a], **Laura A. Gillies**[d], **Luis A.G. Almada**[a], and **Kyria L. Boundy-Mills**[a]

[a]Department of Food Science and Technology, College of Agricultural and Environmental Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA

[b]Forestry Research and Development Agency (FORDA), the Ministry of Forestry, Jalan Gunung Batu No.5, Bogor 16610, Indonesia

[c]Department of the Biosystems Engineering, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

[d]La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037, USA

Abstract

Oleaginous yeasts have been studied for oleochemical production for over 80 years. Only a few species have been studied intensely. To expand the diversity of oleaginous yeasts available for lipid research, we surveyed a broad diversity of yeasts with indicators of oleaginicity including known oleaginous clades, and buoyancy. Sixty-nine strains representing 17 genera and 50 species were screened for lipid production. Yeasts belonged to Ascomycota families, Basidiomycota orders, and the yeast-like algal genus *Prototheca*. Total intracellular lipids and fatty acid composition were determined under different incubation times and nitrogen availability. Thirteen new oleaginous yeast species were discovered, representing multiple ascomycete and basidiomycete clades. Nitrogen starvation generally increased intracellular lipid content. The fatty acid profiles varied with the growth conditions regardless of taxonomic affiliation. The dominant fatty acids were oleic acid, palmitic acid, linoleic acid, and stearic acid. Yeasts and culture conditions that produced fatty acids appropriate for biodiesel were identified.

Keywords

Oleaginous yeast; Biodiesel; Nitrogen-starvation; Triacylglycerides; Oleic acid

1. Introduction

Concerns about climate change and energy independence are driving development of renewable, non-food based liquid transportation fuels such as biodiesel. Biodiesel could reduce global warming impacts; lower emissions of CO, CO_2, sulfur oxide, and particulate

*Corresponding author. Tel.:+81-530-752-1103; fax: +81-530-752-4759. irsitepu@ucdavis.edu; irnars@hotmail.com (I.R. Sitepu)..

matter; is highly degradable and non-toxic; is a drop-in replacement fuel for existing diesel vehicle and boiler engines without major modifications; and presents economic potential for rural growers (Atabani et al., 2012). Edible plant oils used for biodiesel worldwide are rapeseed (84%), sunflower (13%), palm oil (1%), and soybean and others (2%) (Atabani et al., 2012). The rising cost of edible plant oils and public debate of the “food vs. fuel” issue have encouraged development of biodiesel from non-edible plant oils such as jatropha, jojoba, and waste oils such as cooking grease and animal fats. However, these oils may not be sufficiently abundant to meet global needs, and animal fats perform poorly in cold weather.

Biodiesel, the primary renewable alternative to petroleum diesel, is produced by trans-esterification of tryacylglycerides (TAGs) into fatty acid methyl esters. Fatty acid composition has significant impacts on performance of biodiesel (Knothe, 2005; Knothe, 2008; Steen et al., 2010). Chain length, the degree of unsaturation and branching modify cetane number, melting point, oxidative stability, kinematic viscosity and heat of combustion, which are relevant properties that a biodiesel must meet in order to comply with official standards, such as ASTM D6751 & EN 14214 (Knothe, 2008). The relationship between the structural features and the chemical specifications appear in Table 1. Oil sources with high amounts of oleic acid would be ideal candidates for biodiesel purposes, because this fatty acid best meets these criteria (Knothe, 2005).

Microbes including bacteria, fungi (including yeasts), and microalgae have been considered alternate sources of Single Cell Oils (SCO) for oleochemicals including biodiesel. For example, there is currently significant interest in using microbially synthesized TAGs for production of aviation fuels. Microorganisms that are able to accumulate lipids above 20% of their biomass on a dry basis are termed oleaginous (Ratledge, 1979). Oleaginous yeasts exhibit advantages over microalgae in that they have faster duplication times, are relatively easy to scale up, and their production is not subject to seasonal and cyclical weather variations (Ageitos et al., 2011).

Oleaginous yeasts have the capability to synthesize and accumulate high levels of TAGs within their cells, up to 70% of the biomass weight, including for example Lipomyces starkeyi, Rhodosporidium toruloides, Rhodotorula glutinis, and *Yarrowia lipolytica* (Ageitos et al., 2011; Botham and Ratledge, 2011). Examination of enzymatic activity (Ratledge and Wynn, 2002) and comparative genomics (Vorapreeda et al., 2012) suggest that ATP:citrate lyase (ACL) may play a role in directing excess carbon to be stored as lipids rather than carbohydrates in oleaginous yeasts. Analysis of additional oleaginous yeast species, or high- and low-lipid accumulating strains of the same species, could be used to confirm and expand these observations.

Numerous studies have reported oil content and/or fatty acid composition of yeasts (Beopoulos et al., 2010; Kaneko et al., 1976; Meng et al., 2009; Rattray, 1988); however, many of these studies utilized a limited number of species. The fatty acid profiles of most of the 1600 currently known yeast species have not been reported. Additionally, differences in culture conditions make it impossible to compare results from different studies, as oil accumulation depends highly on culture conditions such carbon source, nitrogen source, C/N molar ratio, temperature, and oxygenation (Ageitos et al., 2011). Screens for oleaginous yeasts selected on indicators of oleaginicity such as growth in glycerol (Pan et al., 2009), growth in nitrogen-poor medium (Kraisintu et al., 2010), or strains cited in patents on oil production (Gujjari et al., 2011) had higher success rates in identifying oleaginous strains than screens of yeast strains selected randomly (Kaneko et al., 1976; Kitcha and Cheirsilp, 2011; Rattray, 1988). Therefore, indicators of potential oleaginicity should be used to select yeasts likely to accumulate high amounts of lipids.

Of over 1600 known yeast species, over 40 are known to be oleaginous. These yeasts belong to ascomycete genera Candida, Cyberlindnera, Geotrichum, Kodamaea, Lipomyces, Magnusiomyces, Metschnikowia, Trigonopsis, Wickerhamomyces, and *Yarrowia* (Eroshin and Krylova, 1983; Kitcha and Cheirsilp, 2011; Pan et al., 2009; Rattray, 1988) and to basidiomycete genera Cryptococcus, Guehomyces, Leucosporidiella, Pseudozyma, Rhodosporidium, Rhodotorula and *Trichosporon* (Amaretti et al., 2010; Eroshin and Krylova, 1983; Hansson and Dostalek, 1986; Husain and Hardin, 1952; Pedersen, 1961; Sitepu et al., 2012). Oleaginicity is not clade-specific: oleaginous and non-oleaginous species reside in the same clades. This suggests that oleaginicity arose multiple times independently, and thus may have multiple mechanisms. Some clades, such as the *Rhodotorula glutinis* clade, contain multiple high-oil species as well as lower-oil species. Furthermore, some species such as *Rhodosporidium diobovatum* include both high and low lipid accumulating strains (Sitepu et al., 2012).

The lipid composition and energy value of yeast oils are similar to plant and animal oils, but their production does not compete for food resources, in particular if it is based on inexpensive carbon sources, such as by-products and waste products. Conversion of lignocellulosic hydrolysates to oil by a few oleaginous yeasts has been achieved (Huang et al., 2013; Wang et al., 2012; Yu et al., 2011; Zhan et al., 2013). Expansion of the known oleaginous yeast species and strains would allow selection of yeasts with hydrolysate-specific properties such as carbohydrate utilization, osmotolerance, tolerance of low pH, toxin tolerance, and ability to grow without supplemented vitamins.

High glucose concentrations inhibit growth and lipid accumulation in Crabtree-positive yeasts, but not Crabtree-negative yeasts (Rattray et al., 1975). For example, increasing glucose up to 120 g/L resulted in both increased biomass and increased lipid in *Rhodotorula glacialis*(Amaretti et al., 2010). Oleaginous yeasts able to accumulate both high cell mass and high lipid in high sugar conditions would be useful for conversion of high-sugar lignocellulosic hydrolysates such as AFEX™-pretreated corn stover hydrolysate, which contains over 80 g sugar per liter (Scwalbach et al., 2012).

Here, we report results of intracellular lipid accumulation and fatty acid profiles of 69 yeasts strains representing a broad range of basidiomycetous and ascomycetous yeasts, as well as *Prototheca*, which are non-photosynthetic algae in the order Chlorelalles with cellular and colony morphology similar to yeasts. Strains were selected based on two indicators of oleaginicity: belonging to known oleaginous yeast species or their taxonomic relatives, or buoyancy in 20% glycerol (Sitepu et al., 2012). Three culture conditions were used to obtain additional information on the effect of nitrogen depletion and incubation time on cell biomass accumulation, lipid accumulation and the fatty acid proportions.

2. Material and Methods

2.1 Yeast strains and culture conditions

Sixty-nine (69) yeast strains used in this study were selected from the Phaff Yeast Culture Collection, the University of California Davis (UCDFST). They represented a broad range of Basidiomycota and Ascomycota as well as Viridiplantae order Chlorellales (genus *Prototheca*), a non-photosynthetic alga with yeast-like cells and colonies (Table 2). Yeast strains selected included known oleaginous species, their taxonomic relatives, or buoyant strains. Phaff collection yeasts are routinely cryopreserved by placing cell mass scraped off a PDA agar plate (potato dextrose, Difco™, Sparks, MD, USA), a nitrogen-limiting medium, into a cryovial containing 20% glycerol. Most yeasts sink. Known oleaginous species float, as well as many additional species not previously known to be oleaginous. We

previously confirmed that buoyancy in 20% glycerol is an indicator of oleaginicity (Sitepu et al., 2012).

Yeast strains were revived from cryo-preserved stocks stored at −80 °C and grown on PD agar plates. One colony was inoculated into 10 mL YM broth (yeast extract 3.0 g/L, malt extract 3.0 g/L, peptone 5.0 g/L, dextrose 10 g/L) in a roller-drum at 24 °C for 24 h. This seed culture was transferred to 10 mL modified Medium A (MedA+), making a 5% inoculum concentration and grown in the same condition for 24 h. MedA+ is modified media after Medium A described in Suutari et al. (1993) with 120 g glucose/L (rather than 30), 0.1 g/L calcium chloride, 0.5 g/L ammonium chloride, 1.5 g/L yeast extract (Cat. # 211929, Becton, Dickinson and Company, Sparks, MD, USA), 7.0 g/L KH_2PO_4, 2.5 g/L $Na_2HPO_4{\cdot}2H_2O$, 1.5 $MgSO_4{\cdot}7H_2O$, 0.08 g/L $FeCl_3{\cdot}6H2O$, 10.0 mg/L Zn $SO_4{\cdot}7H_2O$, 0.07 mg/L MnSO $\cdot_4H_2O$, 0.1 mg/L $CuSO_4$, 0.063 mg/L $Co(NO_3)_2$. Three (3.0) mL of this pre-culture were transferred to a 250 mL Erlenmeyer flask containing 125 mL $MedA^+$ (culture condition A), making a 2.4% inoculum concentration and shaken in a rotary shaker incubator (Series 25, New Brunswick Scientific Co., Inc., Edison, New Jersey, USA) at 28 °C, 150 rpm for 72 h to allow exponential growth. Growth was followed by measuring the optical density at 600 nm (OD600) with a spectrophotometer (NanoDrop 2000c UV-Vis spectrophotometer, Thermo Scientific Inc., Wilmington, Delaware, USA), at 0, 24, 48 and 72 h after inoculation. Fifteen (15) mL of these cultures were harvested after 72 hr by centrifugation at 3,220 × g for 10 min, the supernatant was decanted, and the pellet was washed twice with 15 mL sterile deionized water. Cell pellets were stored overnight at −80 °C, lyophilized by freeze-drying (Freezone® 4.5 L Freeze Dry System Model 7750020, Labconco®, Kansas City, Missouri, USA) for 48 h, dry cell was weighed and stored at −80°C. Two (2.0) aliquots of 25 mL of the remaining culture were washed as described previously and resuspended in either $MedA^+$ (culture condition B) or $MedA^+$ without yeast extract or NH_4Cl (no nitrogen, culture condition C). The washed cells were resuspended in 50 mL of the target medium and incubated for 48 h, after which cells were harvested by centrifugation, freeze-dried, weighed, and stored at −80°C as described previously. The initial pH for culture conditions A, B, and C was adjusted to 5.5 with 6N HCl. The cultivation flow chart is presented in Figure 1.

2.2 Determination of total lipid quantity by gravimetric analysis

All chemicals were of analytical grade. Several cell lysis methods were compared including enzymatic, physical, and chemical procedures, and relative lysis determined microscopically. Bead beating was determined to be superior (data not shown). To measure total lipid content, gravimetric analysis was used following Sitepu et al. (2012) as follows. Triplicate 20 mg samples of freeze-dried cells were transferred to 2.0 mL screw cap tubes, with 1.5 mL Folch solvent (2:1 of $CHCl_3$:MeOH, v/v; Folch et al., 1956) (chloroform (Cat.# C2974) and methanol (Cat. # A454-1), Fisher Scientific Inc., Fair Lawn, New Jersey, USA), 0.5 mm zirconia beads (Cat.# 11079105z, Biospec Products Inc., Oklahoma, USA), and two, 3.5 mm glass beads (Cat.# 11079135, Biospec Products Inc., Oklahoma, USA). Cells were homogenized in an MP Bio Fast Prep®-24 homogenizer (MP Biomedicals, Ohio, USA) for 30 sec, 5X with 30 sec interval on ice. A total of 6.0 mL Folch solvent was used to extract lipids and 1.2 mL 0.9% NaCl was added for improved phase separation. Three (3.0) mL of the chloroform-rich phase was evaporated gradually in a 2.0 mL pre-weighed amber vial (Cat.# 500-322, Sun Sri, Rockwood, Tennessee, USA) under a slow stream of nitrogen. The lipid weight after extraction was used to calculate the total lipid as a percent of cell dry weight.

2.3 Determination and quantification of total fatty acid methyl esters by Gas Chromatography-Flame Ionization Detector

This method was based on standardized protocols (Heinze et al., 2012) that were optimized and modified specifically for yeast lipids in this study. The dried lipid extracts were resuspended in 3.0 mL chloroform. 375 μL were transferred into a 2.0 mL pre-weighed amber vial containing a known concentration of internal standard (TAG 17:0 (glyceryl trihepta-decanoate, Cat.# T2151-100mg, Sigma-Aldrich Co. LLC., St. Louis, Missouri, USA) and processed for fatty acid methyl ester (FAME) profiling through trans-esterification. Trans-esterification was performed by adding 1.0 mL of 3N MeOH HCl (methanolic hydrochloric acid, Cat.# 33050-U, Supelco, Sigma-Aldrich Co. LLC., St. Louis, Missouri, USA) and incubating at 60 °C for 14-16 h. Following derivatization, the solution was neutralized at room temperature by adding 500 μL of 6% K_2CO_3 (potassium bicarbonate, Cat.# P184-500, Fisher Scientific, Fair Lawn, New Jersey, USA). To extract the FAMEs, 300 μL of hexane (Cat.# H306-1, Optima grade from Fisher Scientific Inc., Fair Lawn, New Jersey, USA) with 0.01% antioxidant BHT (butylated hydroxytoluene, Cat.# 101162, MP Biomedicals LLC., Solon, Ohio, USA) were added to the solution. After vortexing briefly and centrifuging at 210 × g for 10 min at 4 °C, the hexane layer was transferred to a 0.2 mL GC vial insert (Cat. # 200-238, Sun Sri, Rockwood, Tennessee, USA). Prior to injection to the GC, samples were run on a TLC plate (thin layer chromatography coated with silica gel 60 A, Cat.# 4860-820, Whatmann®International Ltd., Maidstone, England) to check for complete FAME derivatization. TAG 17:0 (glyceryl trihepta-decanoate, Cat.# T2151-100mg, Sigma-Aldrich Co. LLC., St. Louis, Missouri, USA) was used as a reference on the TLC plate to observe the presence of FAMEs and check for complete derivatization.

The fatty acid methyl ester profiling and quantification were performed with a gas chromatograph (Model 7890 GC-FID system, Agilent Techologies) and autosampler (7683 Gas Chromatograph Serial# CN10550113 Santa Clara, California, USA) fitted with a split injector and a flame ionization detector (FID) with nitrogen as carrier gas with a flow of 25 mL/min, equipped with a DB-225ms phase capillary column (30 m length, internal diameter of 0.25 mm with fused-silica column coated with a 0.25 μm polyethylene glycol film) (Cat.# 122-2932, J&W Scientific, Folson, CA, USA). Samples (3.0 μL) were injected using a 10.0 μL syringe and front detector temperature of 280 °C. The carrier gas used was hydrogen at a flow-rate of 35 ml/min, an average velocity of 68 cm/sec and a constant pressure of 17 p.s.i. Air flow was 375 mL/min. Initial temperature was 40 °C and was held for one min after injection, then from 40 to 165 °C at 8.25 °C/min, 165 to 192 °C at 3.75 °C/min, 192 to 197 °C at 16.5 °C/min, 197 to 235 °C at 16.5 °C/min and held for 3 min, 235 to 240 °C at 50 °C/min and held for 3.5 min.

2.4 Data analysis

Peak areas of the fatty acids were processed using Chem32 for Microsoft Windows software (version 1.18, Agilent Technologies Inc., Santa Clara, CA, USA). The fatty acid methyl ester profile of each sample was determined by comparison of the retention time of each FAME peak present in the sample with the retention time of FAME reference standards contained in the reference standard mixture GLC-461 (NuChek Prep, Inc., Elysian, Minnesota, USA). The standard GLC 461 allowed determination of C12 – C24 chain length fatty acids. TAG 17:0 was included in each sample as the internal standard for quantification of fatty acids. Percentage of total fatty acid content was calculated as the ratio of individual FAME peak area to the sum of the all FAME peak areas, excluding the internal standard. The weight of each fatty acid was calculated by multiplying the amount of internal standard (IS) added by the ratio of FAME peak area to the internal standard peak area. These values

were then divided by the dry cell biomass to yield milligrams of fatty acid per gram of dry yeast cells.

3. Results and Discussion

3.1 Cellular mass production varied under different culture conditions

Cell biomass accumulation in culture conditions A, B and C are shown in Table 2. Fifty-one (51) out of 69 yeast strains produced higher cellular mass when grown in culture condition B than those grown in culture conditions A and C. Yeasts that accumulated ≥ 5 g/L dry cellular mass in culture condition B, in comparison with culture conditions A and C are listed in Figure 2. The highest cell accumulation of 10.44 g/L was produced by *Cryptococcus victoriae* UCDFST 10-939 in Culture Condition B, a strain isolated from beetle larva gut in Sulawesi, Indonesia, and the lowest was *Cr. wieringae* UCDFST 05-544 in Culture Condition B, a strain isolated from *Prunus cerasus* nectar in Winters, California, USA. Two strains of the pink-color yeasts *Rhodosporidium babjevae* UCDFST 04-877 and 05-613 were among the highest cellular mass producers (Figure 2). The range of dry cellular mass accumulation in culture condition A was 0.15 – 7.33 g/L culture, and 0.22 - 8.00 g/L in culture condition C. Yeast-like alga *Protetecha* aff. *zopfii* UCDFST 10-495 had the lowest mass in culture condition A. On the other hand, two strains of the genus *Rhodotorula* (*R. aurantica* UCDFST 02-119 and *R. colostri* UCDFST 67-113), one strain of *Rhodosporidium babjevae* UCDFST 04-877, and one strain of *Cryptococcus* cf. *uniguttulatus* UCDFST 68-873, did not show any differences in their cellular mass production in culture conditions B and C (Table 2). Cell accumulations were relatively low, however the accumulation of cell dry weight per liter of culture is consistent with shake flask studies of oleaginous yeasts, which generally accumulate 5-10 g cells/liter (Ageitos et al., 2011; Amaretti et al., 2010; Huang et al., 2013). Culture conditions for each species were not optimized in this study because high cell mass accumulation was not the objective. Future work will include growing the most promising of these strains in stirred, fed-batch fermenters, which would be expected to generate much higher cell biomass.

3.2 Neutral lipid production increased in nitrogen-starved medium

Rattray et al. (1975) summarized observations that for a given yeast strain, the total yeast lipid content and the composition of cellular fatty acids are markedly influenced by variation of growth conditions, including the carbon source, C/N ratio, other nutrients, oxygen level, pH, and temperature. The current study confirms the impact of nitrogen depletion on lipid accumulation, and expands these observations to a broader variety of species.

Lipids such as diacyl- and triacylglycerides, free fatty acids, sterols, carotenoids, and phospholipids are found in both oleaginous and non-oleaginous yeasts (reviewed in Rattray, 1988). Oleaginous yeasts in addition accumulate significantly more triacylglycerides (TAG) in intracellular lipid bodies (reviewed in Rattray, 1988; Rattray et al., 1975). Nitrogen starvation is known to trigger TAG accumulation in most oleaginous yeasts (Rattray, 1988; Rattray et al., 1975). One exception is *Cryptococcus terricola*, which can accumulate TAGs during logarithmic growth, before nitrogen depletion (Pedersen, 1961). This is a valuable property for industrial production, allowing shorter production times and lipid production in nitrogen-rich substrates. Equally high oil production under our culture conditions A and C would suggest that oil accumulation could occur during logarithmic growth.

For non-oleaginous yeasts, when nitrogen is depleted from the medium, protein production and growth stop, and carbon excess is diverted to form polysaccharides (Ageitos et al., 2011). For oleaginous yeast species, however, excess carbon is channeled into lipid bodies in the form of TAGs (Ageitos et al., 2011). In concurrence with these observations, for most

strains analyzed in this study, higher lipid accumulation was observed following the depletion of nitrogen from the medium (culture condition C). It was also observed that high carbon media induced lipid accumulation.

For most yeasts tested in this study, highest lipid levels were obtained in cells grown under nitrogen starvation, represented by culture condition C (Table 2). More than 60% (43 out of 69 strains) of the yeast strains tested in this medium accumulated intracellular lipids to greater than 20% of their cellular dry weight, thus they are oleaginous by definition (Ratledge, 1979). The extractable lipids ranged from 8.81 - 65.32 percent of cell dry weight (%CDW). The strain that accumulated the least extractable lipids was *Myxozyma* cf. *melibiosi* UCDFST 76-318.3. In contrast, the strain that accumulated the most extractable lipids was *Rhodosporidium babjevae* UCDFST 05-775. After three days of growth (culture condition A), the range of lipid accumulation was 8.69 – 50.84% CDW, with 28 of these strains accumulating more than 20% lipid. Continuation of growth in medium containing nitrogen for two additional days (culture condition B) increased the total lipids in a few but not all oleaginous yeasts. Yeasts that can accumulate high levels of oil in three days rather than five are more desirable for industrial scale production.

3.3 Fatty acids proportion and profile

As we observed previously (Sitepu et al., 2012), the total lipid accumulation varied considerably among strains of the same species and the same phenomenon was verified in this study. For example, in culture condition C, *Rhodotorula glutinis* UCDFST 06-542 produced almost three times more total lipids (58.05%) compared to *R. glutinis* UCDFST 50-309 (19.25%) (Figure 3). This underscores the importance of reporting the strain ID number in publications, and selecting strains with known oil accumulation abilities.

While total lipid content can vary greatly within a species, overall fatty acid profiles have been shown to be quite consistent within a species if grown under consistent conditions, and in fact were used to identify and classify yeasts before ribosomal sequencing became affordable (Botha and Kock, 1993). The major fatty acids in most of the yeast strains analyzed were oleic (18:1ω9), palmitic (16:0), stearic (18:0), and linoleic (18:2ω6) acids (Figure 3, Supplementary Tables 1 and 2). Minor fatty acids were lignoceric acid (24:0), palmitic (16:1ω7), behenic acid (22:0), myristic acid (14:0), α-linolenic (18:3ω3), and arachidic acids (20:0). Other fatty acids were observed in trace amounts (data not presented). This is in accordance with fatty acid profiles of yeast species as analyzed by other researchers (Amaretti et al., 2010; Botha and Kock, 1993; Rattray, 1988). No novel or unusual fatty acids were detected.

The effects of nitrogen source and levels on total lipids and on fatty acid profiles have been analyzed with varying results, depending on the yeast species being examined. For instance, in a study of 17 species of yeasts (Evans and Ratledge, 1984), lipid content varied little among most species grown in ammonium chloride, asparagine and glutamate; an exception was *Rhodosporidium toruloides*, which had much higher lipid yields when grown on organic nitrogen than on inorganic nitrogen. *Cryptococcus albidus* generated almost identical growth curves when grown in several nitrogen sources (Hansson and Dostalek, 1986). *Rhodosporidium toruloides* (formerly called *Rhodotorula gracilis*) produced highest lipids and biomass when grown in urea, and lipid productivity was higher in ammonium chloride than in ammonium sulfate (Husain and Hardin, 1952). Further work is needed to determine whether ammonium chloride is the optimal nitrogen source for lipid productivity for each specific yeast. However, regardless of the optimal nitrogen source identified in the laboratory, the nitrogen source for industrial production may be determined by the composition of the lignocellulosic hydrolysate to be utilized. For instance, ammonium is the dominant nitrogen source in AFEX-pretreated corn stover hydrolysate, in sufficient

concentration to support yeast growth (Schwalbach et al., 2012). All known yeasts can utilize ammonium as a nitrogen source. For a given hydrolysate, an oleaginous yeast strain should be selected that can utilize the available nitrogen substrate for growth.

Varying the medium composition and incubation time affected the relative quantities of certain species of fatty acids. For example, the synthesis of linoleic acid (18:2ω6) increased with nitrogen starvation and longer incubation time. This fatty acid species belongs to omega-6 fatty acid group that is an essential fatty acid that cannot be synthesized by humans or animals, but must be ingested for good health. Yeasts that are able to produce significant amounts of omega-6 fatty acids may be considered candidates for commercial production of this fatty acid for nutritional use.

The dominant fatty acid detected was oleic acid (18:1ω9). This species of fatty acid as well as total low polyunsaturated content are two desirable properties of biodiesel. Out of 69 yeast strains tested, six strains produce fatty acids with the desired biodiesel properties including high cell biomass conversion, high lipid production, and the fatty acid profiles. These strains produced the highest levels of oleic acid (C18:1ω9) in two different culture conditions, B and C (Table 3.). Four (4) of the highest strains belong to the same species, *Rhodosporidium babjevae*, in particular, strains UCDFST 04-877 grown in culture condition C and UCDFST 05-775 grown in culture condition B had the highest oleic acid content of 62.92% (234.5 mg/g cellular dry mass) and 61.40% (72.0 mg/g cellular dry mass), respectively.

3.4 Selection of yeast strains for their lipid content

Prior observations of the major factors that affect cellular mass and cell lipid accumulation were supported and expanded by this study. First, as has been observed previously, different yeast species, and strains of those species, have significantly different capacity to accumulate high levels of intracellular TAGs (Rattray, 1988). Second, nitrogen limitation induced accumulation of more TAGs in most of the yeast strains tested in this study.

Our results emphasized that lipid accumulation per cell dry weight cannot be used as the only consideration when selecting specific yeast strains for high lipid production applications. Other factors like cell biomass production must not be overlooked. For example, strain UCDFST 10-939 *Cryptococcus victoriae* produced the highest cell biomass (8 g/L), but accumulated only 19.96% TAGs and could only produce 1.60 g lipid per L culture. On the contrary, UCDFST 05-775 *Rhodosporidium babjevae* produced less than half the biomass of UCDFST 10-939 (3.33 g/L) but accumulated more than twice the TAGs (65.32%) and produced a higher overall lipid accumulation (2.18 g/L), which would generate less cellular waste byproduct after oil extraction, reducing disposal costs.

Optimization of growth conditions is expected to increase both cell and TAG yield when yeasts are grown on a specific substrate. For example, Zhu et al. (2008) recently optimized lipid production by *Trichosporon fermentans.* Starting with a broader palette of oleaginous yeast strain candidates will aid in development of industrial lipid production protocols. Parent strains can be selected with the best baseline properties such as utilization of carbohydrates present in biomass hydrolysates, tolerance of toxins and other stresses, growth temperature, ability to grow without supplemented vitamins, and other properties favorable for industrial fermentations.

3.5 Discovery of new oleaginous yeast species and potential future work

Energy demand in the transportation sector is expected to continue to rise in coming decades. Biodiesel is currently one of the best available alternatives to petroleum, with plant oils currently in use and microbial oils in development (Atabani et al., 2012). Microalgae are

currently the most visible target of microbial oil research, but oleaginous fungi and bacteria are also being studied. A limited number of oleaginous yeast species have been studied in depth (Meng et al., 2009; Rattray et al., 1975) most prominently Yarrowia lipolytica, Lipomyces starkeyi, Cryptococcus curvatus, Rhodotorula glutinis, and *Trichosporon* species. Many additional oleaginous species have been described over the years but not studied thoroughly.

A number of yeast species and strains not previously reported as oleaginous were discovered in this study and are highlighted in Table 2, including: *Myxozyma melibiosi* UCDFST 52-87 (floater), *Kurtzmaniella cleridarum* UCDFST 76-729.2 (floater), *Cryptococcus* aff. *taibaiensis* UCDFST 73-750 (floater), *Rhodotorula colostri* UCDFST 67-113 (oleaginous relative), *Hannaella* aff. *zeae* UCDFST 92-112 (floater), *Tremella enchepala* UCDFST 68-887.2 (floater), *Cr. oeirensis* UCDFST 05-864 (floater), *Cr. terreus* UCDFST 61-443 (floater), *Cr. wieringae* UCDFST 05-544 (floater), *Trichosporon guehoae* UCDFST 60-59 (floater), *Rhodosporidium babjevae* UCDFST 05-775 (floater, oleaginous relative), 04-877 (floating species, oleaginous relative), 68-916.1 (floater, oleaginous relative), 05-736 (floater, oleaginous relative), and 05-613 (floater, oleaginous relative), *R. sphaerocarpum* UCDFST 68-43 (floating species), and *Prototheca* aff. *zopfii* UCDFST 10-495 (floater). We recently reported new oleaginous species *Cryptococcus victoriae* UCDFST 10-939, *Cryptococcus ramirezgomezianus* UCDFST 54-11.224, *R. diobovatum* UCDFST 04-864 and 08-225, *R. paludigenum* UCDFST 09-163 and 82-507.2, *Rhodosporidium fluviale* UCDFST 81-485.4 (Sitepu et al, 2012). Many of these new oleaginous species are basidiomycetes, which are typically capable of utilizing multiple carbon sources. Basidiomycetes are more likely than ascomycetes to be able to grow without supplemented vitamins, reducing processing costs.

We have demonstrated a high success rate of finding novel oleaginous yeasts based on buoyancy on 20% glycerol and taxonomic relatedness with the previously known oleaginous yeast species. Using these two selection criteria, we have discovered 18 new oleaginous yeast species in the last year (Sitepu et al., 2012; this work). These are a significant addition to the 40-plus previously known oleaginous yeast species discovered over the last 90 years of research on high-oil yeasts. This larger set of oleaginous species may make it easier for other researchers to discover novel oleaginous strains in the wild, based on habitat origin or buoyancy.

These new oleaginous yeast strains represent broad taxonomic diversity, and diversity of growth characteristics such as utilization of carbohydrates, growth temperatures, and fatty acid profiles. Table 2 lists the taxonomic placement of these yeasts. Many belong to highly polyphyletic genera such as *Cryptococcus, Rhodotorula* and *Candida.* Our six new oleaginous species in the genus *Cryptococcus* are not closely related to each other; in fact, they reside in three different orders. This inconsistency of yeast systematic is currently being resolved by yeast taxonomists. For example, a clade of former *Cryptococcus* species was recently placed in the new genus *Hannaella* (Wang and Bai, 2008). These taxonomic changes emphasize the importance of citing a strain ID number in publications, as the species name may change, and of depositing strains with promising characteristics into public culture collections.

The first two digits of the UCDFST strain ID number indicate the year that the strain was deposited in the Phaff Yeast Culture Collection. This set of new oleaginous yeasts includes strains isolated and deposited within the last few years, as well as strains maintained for over seven decades, emphasizing the importance of long-term maintenance of biodiversity in biological culture collections for future research. Different levels of lipids accumulation

observed in different strains within the same species emphasize the importance of screening multiple strains of the same species, for this or any other screening project.

In a search for enzymes and metabolites linking glucose to fatty acid synthesis in oleaginous yeasts, Ratledge and colleagues found that ATP:citrate lyase (ACL) is expressed in oleaginous yeasts, but not in non-oleaginous yeasts (Ratledge and Wynn, 2002). Citric acid is used as an acetyl-CoA donor for fatty acid synthesis in the cytoplasm in oleaginous yeasts, and ACL controls the flux of carbon into storage lipids (Botham and Ratledge, 1979). A recent comparative study of oleaginous and non-oleaginous fungal genomes confirmed that genes for enzymes in the ACL pathway are found in the three oleaginous fungi but not the four non-oleaginous fungi studied (Vorapreeda et al., 2012). These types of studies may be supported and expanded in the near future when genome sequences of additional oleaginous yeasts are available. For example, the genome sequence of oleaginous strain *Rhodosporidium toruloides* MTCC457 was recently released (Kumar et al., 2012). Further study of metabolic pathways of additional oleaginous yeasts such as the many new oleaginous species identified in this study will help expand knowledge of the genetic components of oleaginicity.

5. Conclusions

This study revealed many additional novel oleaginous yeast species. After further analysis and strain development, some of these species may prove to be robust production strains for oil production, or as sources of superior genes for manipulation of lipid synthesis pathways in other industrial yeasts. The survey provided extensive preliminary information on the TAG accumulation and fatty acid profiles, and can be used to select yeasts for oleochemicals including biofuels, platform chemicals, and nutritional oils. Further investigation is needed to study the ability of selected oleaginous strains to accumulate lipids under industrially relevant conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Some yeasts used in this study isolated and identified as part of a collaborative project with the Government of the Republic of Indonesia, funded by Grant Number U01TW008160 from the NIH Fogarty International Center, the NIH Office of Dietary Supplements, the National Science Foundation and the Department of Energy. This project was supported by the USDA Agricultural Food Research Initiative of the National Food and Agriculture, USDA, Grant #35621-04750. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Fogarty International Center or the National Institutes of Health, the Office of Dietary Supplements, the National Science Foundation, the Department of Energy, or the Department of Agriculture. Jean VanderGheynst, Yu-Shen Cheng, Hong-Yun Go, Jennifer Smilowitz, Mickael Meyrand, Alejandro Ruiz Ramos, Angela Pratiwiharja, and Marie Stark of the University of California Davis, are thanked for advice or technical assistance. Atit Kanti and Agustinus Joko Nugroho of LIPI Biology, Indonesia; and Sarah Asih Faulina of FORDA, Ministry of Forestry, Indonesia were thanked for the help with isolation of Indonesia microbes. The authors would like to thank the anonymous reviewers for their constructive comments.

REFERENCES

1. Ageitos JM, Vallejo JA, Veiga-Crespo P, Villa TG. Oily yeasts as oleaginous cell factories. Appl. Microbiol. Biotechnol. 2011; 90:1219–1227. [PubMed: 21465305]
2. Amaretti A, Raimondi S, Sala M, Roncaglia L, De Lucia M, Leonardi A, Rossi M. Single cell oils of the cold-adapted oleaginous yeast Rhodotorula glacialis DBVPG 4785. Microbial Cell Factories. 2010; 9:73. [PubMed: 20863365]

3. Atabani A, Silitonga A, Badruddin I, Mahlia T, Masjuki H, Mekhilef S. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. Renew. Sust. Energ. Rev. 2012; 16:2070–2093.
4. Beopoulos, A.; Desfougeres, T.; Sabirova, J.; Nicaud, JM. *Yarrowia lipolytica* as a cell factory for oleochemical biotechnology. In: Timmis, K., editor. Handbook of Hydrocarbon and Lipid Microbiology. Springer-Verlag; Berlin Heidelberg: 2010. p. 3004-3010.
5. Botha A, Kock J. Application of fatty acid profiles in the identification of yeast. Int. J. Food Microbiol. 1993; 19:39–51. [PubMed: 8357755]
6. Botham P, Ratledge C. A biochemical explanation for lipid accumulation in Candida 107 and other oleaginous micro-organisms. J. Gen. Microbiol. 1979; 114:361–375. [PubMed: 44315]
7. Eroshin V, Krylova N. Efficiency of lipid synthesis by yeasts. Biotechnol. Bioeng. 1983; XXV: 1693–1700. [PubMed: 18551475]
8. Evans C, Ratledge C. Effect of nitrogen source on lipid accumulation in oleaginous yeasts. J. Gen. Microbiol. 1984; 130:1693–1704.
9. Gujjari P, Suh S-O, Coumes K, Zhou J. Characterization of oleaginous yeasts revealed two novel species: *Trichosporon cacaoliposimilis* sp. nov. and *Trichosporon oleaginousus* sp. nov. Mycologia. 2011; 103:1110–1118. [PubMed: 21558504]
10. Hansson L, Dostalek M. Effect of culture conditions on fatty acid composition on lipids produced by the yeast *Cryptococcus albidus var. albidus*. J. Am. Oil Chemists. Soc. 1986; 63:1179–1184.
11. Heinze C, Hawkins M, Gillies L, Wu X, Walzem R, German J, Klasing K. Effect of dietary omega-3 fatty acids on red blood cell lipid composition and plasma metabolites in the cockatiel, *Nymphicus hollandicus*. J. of Animal Sci. 2012; 90:3068–3079.
12. Huang C, Chen X-F, Xiong L, Chen X-D, Ma L-L, Chen Y. Single cell oil production from low-cost substrates: The possibility and potential of its industrialization. Biotechnol. Adv. 2013; 31:129–139. [PubMed: 22960618]
13. Husain S, Hardin M. Influence of carbohydrate and nitrogen sources upon lipid production by certain yeasts. J. Food Sci. 1952; 17:60–66.
14. Kaneko H, Hosohara M, Tanaka M, Itoh T. Lipid Composition of 30 Species of Yeast. Lipids. 1976; 11:837–844. [PubMed: 1011938]
15. Kitcha S, Cheirsilp B. Screening of oleaginous yeasts and optimization for lipid production using crude glycerol as a carbon source. Energy Procedia. 2011; 9:274–282.
16. Knothe G. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process. Technol. 2005; 86:1059–1070.
17. Knothe G. “Designer” Biodiesel: Optimizing Fatty Ester Composition to Improve Fuel Properties. Energ. Fuels. 2008; 22:1358–1364.
18. Kraisintu P, Yongmanitchai W, Limtong S. Selection and optimization for lipid production of a newly isolated oleaginous yeast, *Rhodosporidium toruloides* DMKU3-TK16. Kasetsart. J. Nat. Sci. 2010; 44:436–445.
19. Kumar S, Kushwaha H, Kumar Bachhawat A, Singh Raghava G, Ganesan K. Genome sequence of the oleaginous red yeast *Rhodosporidium toruloides* MTCC 457. Eukaryot. Cell. 2012; 11:1083–1084.
20. Meng X, Yang J, Xu X, Zhang L, Nie Q, Xian M. Biodiesel production from oleaginous microorganisms. Renew. Energ. 2009; 34:1–5.
21. Pan L-X, Yang D-F, Shao L, Li W, Chen G-G, Liang Z-Q. Isolation of the oleaginous yeasts from the soil and studies of their lipid-producing capacities. Food Technol. Biotechnol. 2009; 47:215–220.
22. Pedersen T. Lipid formation in *Cryptococcus terricolus*. Acta Chem. Scand. 1961; 15:651–662.
23. Ratledge C. Resources Conservation By Novel Biological Processes. Part 1: Grow fats from wastes. Chem. Soc. Rev. 1979; 8:283–296.
24. Ratledge C, Wynn J. The biochemistry and molecular biology of lipid accumlation in oleaginous microorganisms. Adv. Appl. Microbiol. 2002; 51:1–44. [PubMed: 12236054]
25. Rattray, J. Yeast. In: Ratledge, C.; Wilkinson, S., editors. Microbial lipids. Vol. 1. Academic Press; London: 1988. p. 555-697.

26. Rattray J, Scheibeci A, Kidby D. Lipids of Yeasts. Bacteriolog. Rev. 1975; 39:197–231.
27. Schwalbach M, Keating D, Tremaine M, Marner W, Zhang Y, Bothfeld W, Higbee A, Grass J, Cotten C, Reed J, da Costa Sousa L, Jin M, Balan V, Ellinger J, Dale B, Kiley P, Landick R. Complex physiology and compound stress responses during fermentation of alkali-pretreated corn stover hydrolysate by an Escherichia coli ethanologen. Appl. Environ. Microbiol. 2012; 78:3442–3457. [PubMed: 22389370]
28. Sitepu I, Ignatia L, Franz A, Wong D, Faulina S, Tsui M, Kanti A, Boundy-Mills K. An improved high-throughput Nile red fluorescence assay for estimating intracellular lipids in a variety of yeast species. J. Microbiolog. Methods. 2012
29. Steen E, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, del Cardayre S, Keasling J. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature. 2010; 463:559–562. [PubMed: 20111002]
30. Vorapreeda T, Thammarongtham C, CHeevadhanarak S, Laoteng K. Alternative routes of acetyl-CoA synthesis identified by comparative genomic analysis: involvement in the lipid production of oleaginous yeast and fungi. Microbiol. 2012; 158:217–228.
31. Wang Q, Guo F-J, Rong Y-J, Chi ZM. Lipid production from hydrolysate of cassava starch by *Rhodosporidium toruloides* 21167 for biodiesel making. Renew. Energ. 2012; 46:164–168.
32. Wang QM, Bai FY. Molecular phylogeny of basidiomycetous yeasts in the *Cryptococcus luteolus* lineage (Tremellales) based on nuclear rRNA and mitochondrial cytochrome b gene sequence analyses: proposal of *Derxomyces* gen. nov. and *Hannaella* gen. nov., and description of eight novel *Derxomyces* species. FEMS Yeast Res. 2008; 8:799–814. [PubMed: 18616607]
33. Yu X, Zheng Y, Dorgan K, Chen S. Oil production by oleaginous yeasts using the hydrolysate from pretreatment of wheat straw with dilute sulfuric acid. Bioresour. Technol. 2011; 102:6134–6140. [PubMed: 21463940]
34. Zhan J, Lin H, Shen Q, Zhou Q, Zhao Y. Potential utilization of waste sweetpotato vines hydrolysate as a new source for single cell oils production by Trichosporon fermentans. Bioresour. Technol. 2013 In press.
35. Zhu L, Zong M, Wu H. Efficient lipid production with Trichosporon fermentans and its use for biodiesel preparation. Bioresour. Technol. 2008; 99:7881–7885. [PubMed: 18394882]

- The first study to profile FA from diverse yeasts grown in three culture conditions
- Discovery of thirteen new oleaginous yeast species
- FA proportion varied with the growth conditions regardless of taxonomic affiliation
- Dominant fatty acid were oleic acid, palmitic acid, linoleic acid and stearic acid
- Nitrogen starvation boosted accumulation of linoleic acid in many species

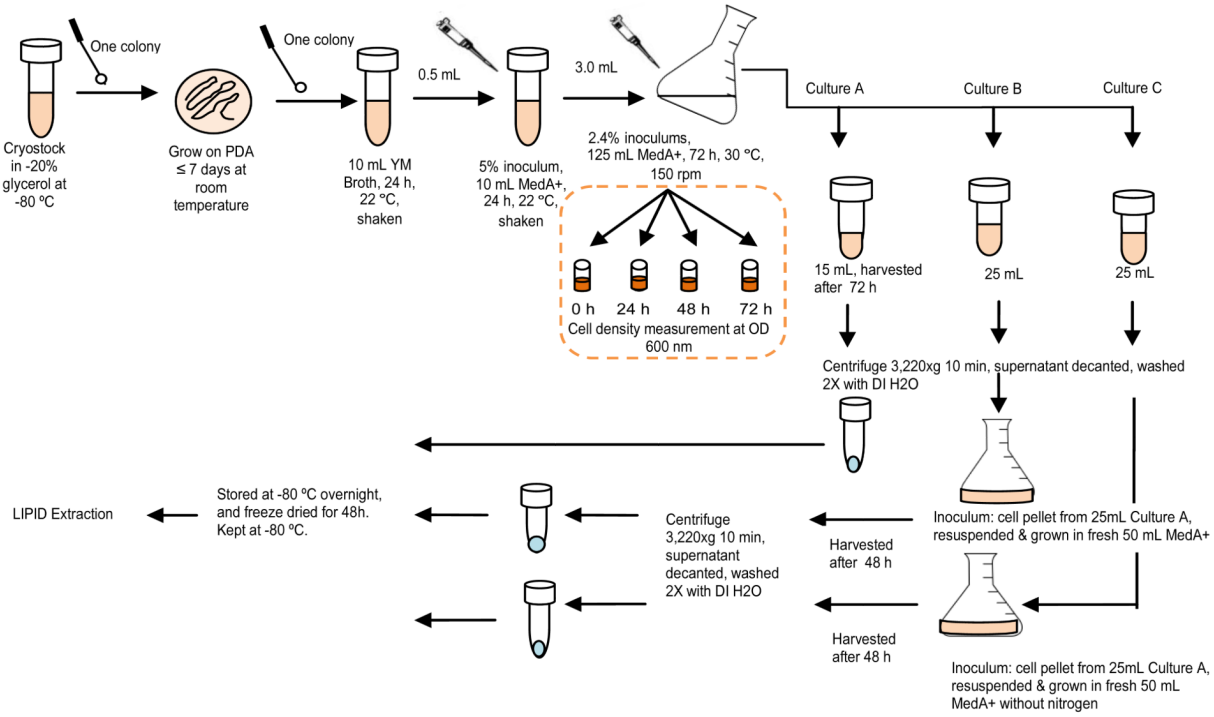

Figure 1.
Cultivation flow chart

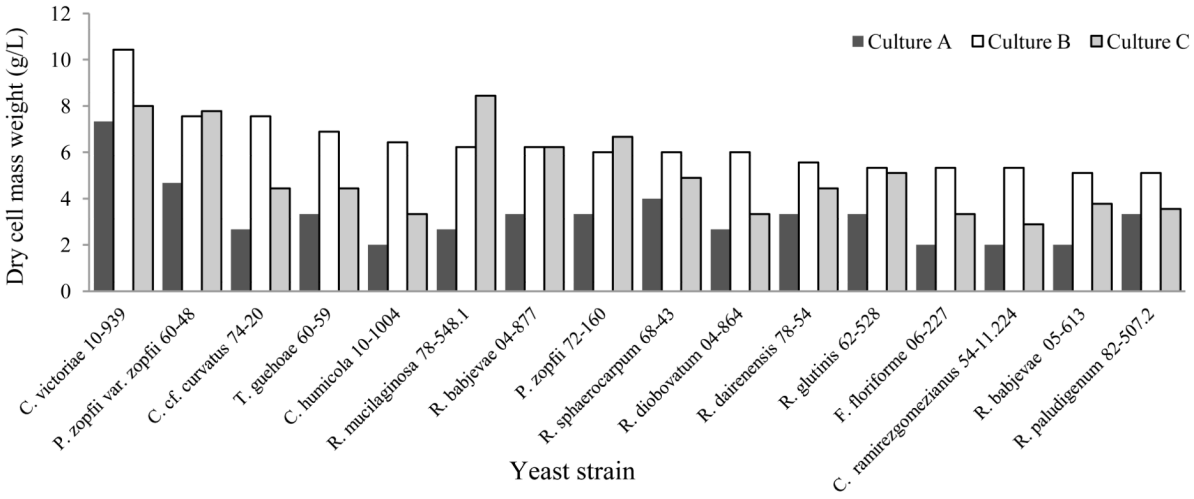

Figure 2.
Yeast strains that accumulated ≥ 5 g/L dry cellular mass in culture B compared with Culture A and C.

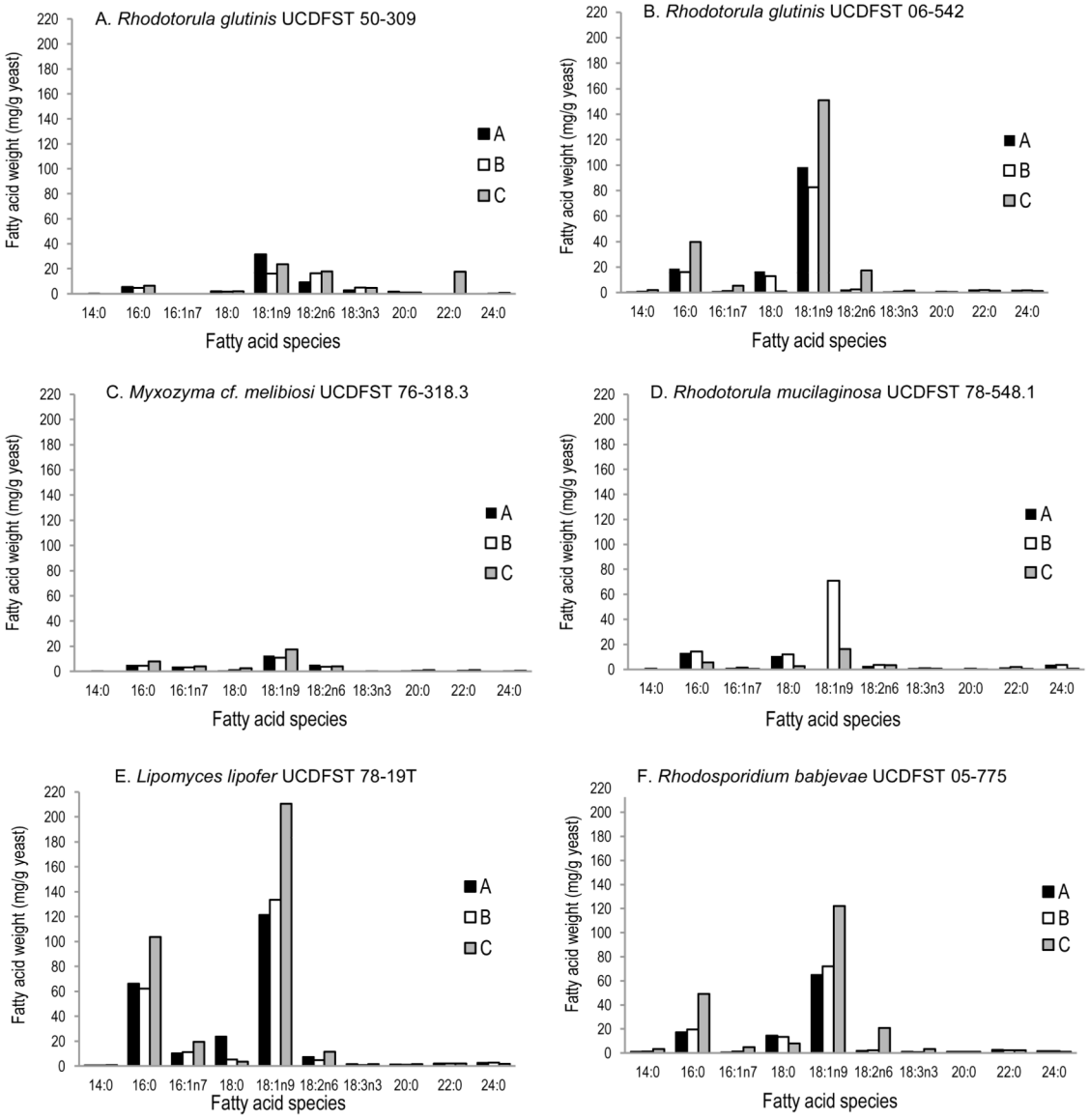

Figure 3.

Comparison of fatty acid profiles of selected yeast strains grown in culture conditions A, B and C as determined by GC-FID, demonstrating intra- and inter-species variation, total lipid content of high and low lipid strains, and the effects of growth conditions on fatty acid profile of a given strain. A. *Rhodotorula glutinis* UCDFST 50-309 with lower (19.25%) lipid content and B. *R. glutinis* UCDFST 06-542 with higher (58.05%) lipid content in culture condition C; C. Low lipid producer *Myxozyma* cf. *melibiosi* UCDFST 76-318.3 (8.81%) and D. *Rhodotorula mucilaginosa* UCDFST 78-548.1 (8.83%) in culture condition C; E and F. Two among the highest lipid producers *Lipomyces lipofer* UCDFST 78-19T (51.33%) and *Rhodosporidium babjevae* UCDFST 05-775 (65.32%) in culture condition C.

Table 1

Relationship between fatty acid structure and biodiesel performance parameters (Knothe, 2005; Knothe, 2008; Steen, 2010).

	Cetane Number (ignition quality: greater is better)	**Melting Point (lower is better)**	**Oxidative Stability (more stable is better)**	**Kinematic Viscosity (less viscous is better)**	**Heat of combustion (greater is better)**
Chain length	Longer is better	Shorter is better	NR	Shorter is better	Longer is better
Degree of unsaturation	Saturated is better	Unsaturated is better	Saturated is better	Unsaturated is better	NR
Branching	NR	Branching is better	NR	NR	NR

Note. NR: Not relevant

Table 2

Strain species and UCDFST ID number, cell dry weight, total intracellular lipids weight and percentage of 69 yeast strains grown in three different culture conditions diagrammed in Figure 1, grouped by their taxonomic affiliations.

A. Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales

Order[a]-Genus species Culture Condition	Other culture collection ID	Source habitat	Cell dry weight (g/L)			Total lipids (%)			Total lipid weight (g/L)		
			A	B	C	A	B	C	A	B	C
Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales											
Debaryomycetaceae											
Scheffersomyces stipitis 10-948	FORDA-CC 776	Fungal fruiting body, Sulawesi, Indonesia	0.65	1.11	0.89	16.06	15.44	17.87	0.10	0.17	0.16
Scheffersomyces stipitis 10-950	FORDA-CC 778	Swab of beetle larva tunnel in beetle-infested log, Sulawesi, Indonesia	0.67	1.56	1.11	15.14	13.30	26.00	0.10	0.21	0.29
Kurtzmaniella cleridarum 76-729.2	-	Mushroom, Patrick Point, California USA	2.00	4.44	2.00	32.24	26.04	33.33	0.64	1.16	0.67
Candida aff. *insectorum* 10-850	FORDA-CC 685	Lepidoptera larva gut, Sulawesi, Indonesia	2.00	3.33	3.11	13.75	15.01	13.98	0.28	0.50	0.43
Lipomycetaceae											
Lipomyces lipofer 78-19T	NRRL Y-11555; CBS 944	Soil	1.33	4.89	2.89	35.95	33.58	51.33	0.48	1.64	1.48
Lipomyces starkeyi 78-23		*Opuntia stricta* Yarrawonga, New South Wales, Australia	2.00	4.67	0.89	25.29	37.48	40.00	0.51	1.75	0.36
Myxozyma melibiosi 52-87	CBS 2102; ATCC 24226; NRRL Y-11781	Bark beetle *Dendroctonus monticolae* in *Pinus ponderosa*, California, USA	2.00	4.67	3.33	19.51	23.44	17.67	0.39	1.09	0.59
Myxozyma cf. *melibiosi* 76-318.3	-	Agria cactus *Stenocereus gummosus*, Baja California Sur, Mexico	2.67	4.22	3.11	9.42	9.67	8.81	0.25	0.41	0.27
Myxozyma mucilagina 76-214.2	-	Organ pipe cactus *Stenocereus thurberi*, Baja California Sur, Mexico	2.00	4.67	3.11	11.13	12.06	20.82	0.22	0.56	0.65
Metschnikowiaceae											
Candida aff. *sagamina* 10-1002	FORDA-CC 577	Liquid in cup fungus, Sulawesi, Indonesia	1.33	2.00	1.11	14.41	12.85	12.77	0.19	0.26	0.14
Pichiaceae											
Pichia manshurica 49-14	-	Olive brine, California, USA	2.00	4.00	2.44	16.00	17.68	15.40	0.32	0.71	0.38
Trichomonascaceae											
Candida aff. *kazuoi* 10-784	FORDA-CC 627	Fungus under bark, Sulawesi, Indonesia	0.67	1.11	0.89	13.17	12.75	10.44	0.09	0.14	0.09
Incertae sedis											
Yarrowia lipolytica 68-653.4	-	Exudate of *Betula papyrifera* Kenai Peninsula, Alaska, USA	2.67	4.44	1.56	8.69	17.63	18.00	0.23	0.78	0.28

B. Basidiomycota

Order[a]-Genus species Culture Condition	Other culture collection ID	Source habitat	Cell dry weight (g/L)			Total lipids (%)			Total lipid weight (g/L)		
			A	B	C	A	B	C	A	B	C
Basidiomycota											
Pucciniomycotina; Cystobasidiomycetes; Aurantiaca group											
Rhodotorula aurantiaca 02-119	-	White alder, Vacaville, California, USA	2.00	1.33	1.33	16.03	20.00	20.51	0.32	0.27	0.27
Pucciniomycotina; Microbotryomycetes; Leucosporidiales											
Leucosporidiella creatinivora 62-1032	-	Exudate of alder tree, California, USA	2.00	3.56	3.78	25.64	21.08	48.58	0.51	0.75	1.84
Pucciniomycotina; Microbotryomycetes; Sporidiobolales											
Rhodotorula colostri 67-113	-	Water from Tomales Bay near Inverness, California, USA	0.67	0.44	0.44	26.89	14.11	18.07	0.18	0.06	0.08
Rhodotorula dairenensis 72-233	-	Fermented arrow root liquid, California, USA	2.67	4.00	2.22	18.82	17.74	21.75	0.50	0.71	0.48
Rhodotorula dairenensis 78-54	-	Wisconsin, USA	3.33	5.56	4.44	16.80	16.92	17.11	0.56	0.94	0.76
Rhodotorula glutinis 06-542	-	Olive fly *Batrocera oleae*-infested olive, Winters, California, USA	1.33	2.89	1.78	40.95	52.19	58.05	0.55	1.51	1.03
Rhodotorula glutinis 62-106		Wasp nest.	2.67	4.44	7.11	22.22	25.34	20.38	0.59	1.13	1.45
Rhodotorula glutinis 50-309	CBS 20; ATCC 2527	Atmosphere, Japan	1.33	1.33	0.67	18.75	19.68	19.25	0.25	0.26	0.13
Rhodotorula glutinis 62-528	-	Susan River water, California, USA	3.33	5.33	5.11	33.75	18.16	22.95	1.12	0.97	1.17
Rhodotorula graminis 62-354	-	Vetch flowers, Saskatoon, Canada	2.00	4.44	4.00	11.44	10.94	21.22	0.23	0.49	0.85
Rhodotorula mucilaginosa 40-129	-	Soil, California, USA	2.00	2.89	3.11	20.87	20.76	32.74	0.42	0.60	1.02
Rhodotorula mucilaginosa 78-548.1	-	*Opuntia stricta*, Grandchester Hill, Queensland, Australia	2.67	6.22	8.44	18.96	20.24	8.83	0.51	1.26	0.75
Rhodosporidium babjevae 05-775	-	Sap on *Olea europaea*, Winters, California, USA	2.00	4.44	3.33	50.84	58.04	65.32	1.02	2.58	2.18
Rhodosporidium babjevae 04-877	-	Female olive fly *Batrocera oleae*, Davis, California, USA	3.33	6.22	6.22	38.78	40.07	46.87	1.29	2.49	2.92
Rhodosporidium babjevae 68-916.1	-	Insect frass of *Alnus* sp. (alder), Emory Creek, British Columbia, Canada	2.00	3.33	3.56	22.58	24.00	35.00	0.45	0.80	1.24
Rhodosporidium babjevae 05-736	-	Female olive fly *Batrocera oleae*, Davis, California, USA	2.00	3.33	4.44	29.10	37.29	43.20	0.58	1.24	1.92
Rhodosporidium babjevae 05-613	-	Female olive fly *Batrocera oleae*, Davis, California, USA	2.00	5.11	3.78	27.08	39.77	40.62	0.54	2.03	1.53
Rhodosporidium diobovatum 04-864	-	Male olive fly, Cruess hall olive trees, Davis, California	2.67	6.00	3.33	21.98	24.21	26.74	0.59	1.45	0.89

B. Basidiomycota

Order[a]-Genus species Culture Condition	Other culture collection ID	Source habitat	Cell dry weight (g/L)			Total lipids (%)			Total lipid weight (g/L)		
			A	B	C	A	B	C	A	B	C
Rhodosporidium diobovatum 08-225	CBS 6085	Sea water, southeastern Florida, USA	4.67	4.44	3.78	35.46	34.51	39.69	1.65	1.53	1.50
Rhodosporidium fluviale 81-485.4	-	*Opuntia ficus-indica*, City of Tucson, Arizona, USA	1.33	3.78	1.33	15.02	14.19	14.97	0.20	0.54	0.20
Rhodosporidium paludigenum 09-163	CBS 3044	Leaf of *Desmodium repens*, The Netherlands	2.00	4.67	3.56	19.79	25.70	39.73	0.40	1.20	1.41
Rhodosporidium paludigenum 82-507.2	-	*Stenocereus hystrix*, Hatillo, Dominican Republic, Caribbean	3.33	5.11	3.56	18.12	17.88	36.32	0.60	0.91	1.29
Rhodosporidium sphaerocarpum 68-43	AU-1	Unknown	4.00	6.00	4.89	25.95	14.81	36.60	1.04	0.89	1.79
Rhodosporidium toruloides 68-264	CBS 315	Air, Tokyo, Japan	2.67	4.44	3.78	27.06	26.00	45.67	0.72	1.16	1.73
Agaricomycotina; Tremellomycetes; Cuniculitremaceae											
Cuniculitrema polymorpha 82-13		Gelatinous deposits on drenched pitwood planks in old ore mine, Germany	0.67	2.44	2.00	15.90	14.58	20.88	0.11	0.36	0.42
Agaricomycotina; Tremellomycetes; Filobasidiales											
Filobasidium floriforme 06-227	-	*Drosophila melanogaster*-infested apple, Davis, California, USA	2.00	5.33	3.33	19.51	16.17	25.72	0.39	0.86	0.86
Filobasidium floriforme 71-71	CBS 6240	Florets of *Eryanthus giganteus* (plume grass), USA	2.00	4.00	2.89	8.87	9.67	13.89	0.18	0.39	0.40
Filobasidium globisporum 05-541		*Prunus cerasus* Shubinka, Wolfskill Experimental Orchard, Winters, CA	2.00	3.56	2.22	14.96	18.82	20.26	0.30	0.67	0.45
Filobasidium aff. *globisporum* 83-617.3	-	Washing from barrel cactus extrafloral nectary	0.67	3.11	1.33	12.30	9.82	11.94	0.08	0.31	0.16

C. Basidiomycota,

Order[a]-Genus species Culture Condition	Other culture collection ID	Source habitat	Cell dry weight (g/L)			Total lipids (%)			Total lipid weight (g/L)		
			A	B	C	A	B	C	A	B	C
Filobasidium inconspicuum 89-39	K. Wells 304-2		1.33	2.67	4.00	12.98	14.11	29.32	0.17	0.38	1.17
Cryptococcus albidus 63-203	-	Fales hot spring effluent, Mono Lake, California, USA	2.00	3.78	1.78	30.40	26.05	33.97	0.61	0.98	0.60
Cryptococcus albidus 76-324.2	-	Cardon cactus (*Pachycereus pringlei*) active rot with *Drosophila* larvae, Baja California Sur, Mexico	2.00	3.56	2.89	10.20	11.19	18.01	0.20	0.40	0.52
Cryptococcus gastricus 81-46	-	Black rot of *Cephalocereus* sp. Cat Island, Bahamas, Caribbean	0.67	2.44	4.00	15.69	12.87	20.46	0.10	0.31	0.82

C. Basidiomycota,

Order[a]-Genus species Culture Condition	Other culture collection ID	Source habitat	Cell dry weight (g/L)			Total lipids (%)			Total lipid weight (g/L)		
			A	B	C	A	B	C	A	B	C
Cryptococcus magnus 10-900	FORDA-CC 733	Cerambycid larva gut, Sulawesi, Indonesia	0.67	0.89	0.44	13.78	17.87	21.18	0.09	0.16	0.09
Cryptococcus oeirensis 05-864	-	Female olive fly *Batrocera oleae*, Davis, California, USA	1.33	3.11	1.78	16.59	16.75	25.79	0.22	0.52	0.46
Cryptococcus terreus 61-443	-	Soil, California, USA	2.00	3.11	3.78	25.44	23.89	51.68	0.51	0.74	1.95
Cryptococcus aff. *taibaiensis* 73-750	-	Freycin leaf axil, Lanai, Hawaii, USA	2.00	2.89	2.44	12.57	10.86	37.37	0.25	0.31	0.91
Cryptococcus flavescens 76-773.1		*Opuntia* (cactus) rot	2.00	3.78	3.56	13.80	13.10	22.55	0.28	0.49	0.80
Cryptococcus flavescens 76-106		Frozen fruit sample, Davis, California, USA	2.67	4.00	3.33	12.08	11.50	16.58	0.32	0.46	0.55
Cryptococcus aff. *laurentii* 68-684.1		Exudate of *Populus tremuloides* at Delta Junction, Alaska, USA	0.15	1.56	0.22	31.30	12.49	17.84	0.05	0.19	0.04
Cryptococcus luteolus 82-434		*Opuntia stricta v. dilennii*, Fond Parisienne, Haiti	0.67	2.67	2.22	13.28	13.39	20.70	0.09	0.36	0.46
Filobasidium cf. *uniguttulatus* 68-873		Frass and beetles attacking a *Picea* sp., near Prince George, British Columbia, Canada	0.67	2.00	2.00	9.84	13.69	20.28	0.07	0.27	0.41
Cryptococcus victoriae 10-939		Beetle larva gut, Sulawesi, Indonesia	7.33	10.44	8.00	11.99	17.69	19.96	0.88	1.85	1.60
Hannaella aff. *zeae* 92-112	-	Rotten bamboo shoot of *Phyllostachys pubescens*. Hseto Park, Taiwan	0.67	2.44	2.22	12.07	12.98	25.59	0.08	0.32	0.57
Tremella aurantia 61-383	-	Insect frass in apple tree attacked by *Scolytus rugulosus*, California , USA	2.67	4.00	4.89	12.55	13.83	14.06	0.33	0.55	0.69
Tremella enchepala 68-887.2	-	Cracked bark of *Salix* sp,. near Prince George, British Columbia, Canada	2.00	4.67	4.00	13.57	17.33	41.67	0.27	0.81	1.67
Agaricomycotina; Tremellomycetes; Trichosporonales											
Cryptococcus cf. *curvatus* 74-20		Unknown	2.67	7.56	4.44	18.15	10.19	19.40	0.48	0.77	0.86
Cryptococcus humicola 10-1004		Fungal Fruiting Body, Sulawesi, Indonesia	2.00	6.44	3.33	26.31	27.45	35.48	0.53	1.77	1.18
Cryptococcus ramirezgomezianus 54-11.224		Fruiting body of *Pleurotus* fungus, California, USA	2.00	5.33	2.89	31.79	29.37	40.05	0.64	1.57	1.16
Cryptococcus wieringae 05-544	-	*Prunus cerasus* nectar, Wolfskill Experimental Orchard, Winters, California, USA	0.67	0.22	0.44	23.15	32.10	52.65	0.15	0.07	0.23
Trichosporon guehoae 60-59	-	Slime flux of a chestnut tree, Winschoten, Netherlands	3.33	6.89	4.44	23.20	21.31	37.48	0.77	1.47	1.67

C. Basidiomycota,

Order[a]-Genus species Culture Condition	Other culture collection ID	Source habitat	Cell dry weight (g/L)			Total lipids (%)			Total lipid weight (g/L)		
			A	B	C	A	B	C	A	B	C
Viridiplantae; Chlorellales; Chlorellaceae											
Prototheca aff. *zopfii* 10-495	FORDA-CC 1911	Cerambycid larva, Sulawesi, Indonesia	0.15	0.67	0.44	46.97	13.08	10.16	0.07	0.09	0.05
Prototheca stagnora 91-462.4	-	*Trichocereus pasacanna*, Tucuman, Argentina	2.00	4.89	2.00	26.07	11.21	14.88	0.52	0.55	0.30
Prototheca zopfii 72-160	-	*Myoporum sandwicense* flux. Ahumoa, Island of Hawaii, USA	3.33	6.00	6.67	14.16	13.91	15.19	0.47	0.83	1.01
Prototheca aff. *zopfii var. hydrocarbonea* 10-493	FORDA-CC 1909	Cerambycid larva, Sulawesi, Indonesia	0.17	0.67	0.22	38.46	14.82	17.61	0.07	0.10	0.04
Prototheca zopfii var. zopfii 60-48	NRRL YB-4826		4.67	7.56	7.78	12.44	16.01	13.37	0.58	1.21	1.04

[a]Taxon Order is indicated in bold. Culture condition A: Yeast cells grown 72 hours in Medium A+. Culture condition B: Regrowth in Medium A+, for an additional 48 h. Culture condition C: Reg Medium A+ lacking nitrogen (yeast extract and NH_4Q), and grown 48 h. All cultures were harvested, washed, cell pellets stored overnight at −80°C, freeze-dried, and maintained at −80°C. UCDFST:Phaff Yeast Culture Collection, Food Science and Technology, University of California Davis, CA, USA; FORDA-CC: Indonesian Ministry of Forestry Research and Development Agency Culture Collection, Bogor, Indonesia; NRRL, USDA-ARS Culture Collection, Peoria, IL, USA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ATCC, American Type Culture Collection, Manassas, VA, USA.

Table 3

Selected yeast strains that display potential properties for biodiesel purposes

Rank	Species, UCDFST ID#	Culture condition	Total Lipid (g/L)	Oleic acid (mg/g)	Oleic acid (%)	Saturates (%)	Polyunsaturates (%)
1	*Rhodosporidium babjevae* 04-877	C	2.92	234.5	62.92	27.35	5.24
2	*Rhodosporidium babjevae* 05-775	B	2.58	72.0	61.40	33.46	2.97
3	*Rhodosporidium babjevae* 05-613	B	2.03	87.0	59.79	32.67	2.40
4	*Rhodosporidium babjevae* 05-736	C	1.92	154.9	57.46	27.00	10.76
5	*Rhodosporidium sphaerocarpum* 68-43	C	1.79	114.7	52.86	31.97	13.68
6	*Cryptococcus terreus* 61-443	C	1.95	134.5	46.72	35.84	13.90