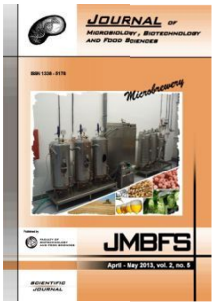

JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

Sriwongchai et al. 2013 : 2 (5) 2344-2348

SCREENING OF SELECTED OLEAGINOUS YEASTS FOR LIPID PRODUCTION FROM GLYCEROL AND SOME FACTORS WHICH AFFECT LIPID PRODUCTION BY *YARROWIA LIPOLYTICA* STRAINS

Salinee Sriwongchai[1], Prayad Pokethitiyook[1], Maleeya Kruatrachue[1], Paramjit K. Bajwa[2] and Hung Lee[2,]**

Address(es): Salinee Sriwongchai and Hung Lee
[1] Faculty of Science and Social Science, Burapha University Sakaeo Campus, Sakaeo, 27160 THAILAND.
[2] School of Environmental Sciences, University of Guelph, Guelph, Ontario, CANADA N1G 2W1.

*Corresponding author: salinee@buu.ac.th and hlee@uoguelph.ca

ARTICLE INFO

Received 3. 11. 2012
Revised 9. 3. 2013
Accepted 11. 3. 2013
Published 1. 4. 2013

Regular article

OPEN ACCESS

ABSTRACT

The ability of eight yeast strains to utilize glycerol as a sole carbon source and accumulate lipids in a chemically defined medium was screened. Among the yeasts, *Yarrowia lipolytica* strains DSM 70561 and JDC 335 grew to high cell densities on glycerol. These strains were further tested for lipid accumulation under varying nutritional conditions in Erlenmeyer flasks. The results showed that strains DSM 70561 and JDC 335 accumulated lipids up to 37.1 % and 54.4 % of total cell dry weight, respectively, when the defined medium was supplemented with 1 g/L urea and 2 g/L yeast extract. The lipids accumulated by the two yeasts contained a high proportion of C16:0, C18:1, C18:2 and C18:0 fatty acids. The results suggest that *Y. lipolytica* strains DSM 70561 and JDC 335 have the potential for converting crude glycerol into fatty acids which can in turn be utilized as substrate for biodiesel production.

Keywords: Glycerol, lipid accumulation, oleaginous yeasts, *Yarrowia lipolytica*, yeast screening

INTRODUCTION

Biodiesel is made from various vegetable oils and animal fats through trans-esterification (Easterling *et al.,* 2009). It is one of the alternative and eco-friendly fuels because of its low toxicity, biodegradability, and low concentrations of small particulate matter and SO_2 in exhaust gas when used in motor vehicles (Ito *et al.,* 2005). The principal by-product of biodiesel production is crude glycerol. It has been estimated that for every 10 kg of biodiesel fuel made from various oils, about 1 kg of crude glycerol is produced (Easterling *et al.,* 2009; Papanikolaou and Aggelis, 2002).

Crude glycerol possesses limited value because of the impurities present. Conversion of crude glycerol to specific products may provide credit to offset the cost of biodiesel production. Crude glycerol can be converted by microorganisms into value added products such as 1,3-propanediol by *Clostridium acetobutylicum* (Gonzalez-Pajuelo *et al.,* 2004) and *C. butyricum* (Asad-ur-Rehman *et al.,* 2008), lipids by oleaginous microorganisms (Easterling *et al.,* 2009; Narayan *et al.,* 2005; Papanikolaou *et al.,* 2003), citric acid and erythritol by *Yarrowia lipolytica* Wratislavia K1 (Rymowicz *et al.,* 2006), and a mixture of succinic acid, butanol, ethanol, and hydrogen by *Escherichia coli* (Dharmadi *et al.,* 2006).

Single cell oil (SCO) refers to the lipids produced by oleaginous microorganisms such as bacteria, yeasts, moulds and algae that can accumulate oils to more than 20 % of their total cell dry weight. Some oleaginous yeasts such as *Rhodotorula* sp., *Rhodosporidium* sp., *Yarrowia lipolytica* (Zhao *et al.,* 2008) and *Cryptococcus curvatus* ATCC 20509 (Ratledge, 1991) were reported to accumulate intracellular lipids to as high as 50 % of their cell dry weight. This was typically achieved during growth on nitrogen-limited media with various carbon sources such as sugars, organic acids, hydrocarbons, fats and vegetable oils, and glycerol or crude glycerol (Li *et al.,* 2007; Papanikolaou *et al.,* 2009; Papanikolaou and Aggelis, 2002; Ratledge, 1991). Although lipid accumulation from glycerol and crude glycerol by selected oleaginous yeasts has been studied, to our knowledge, there has been no study on comparative screening of various oleaginous yeasts for lipid accumulation from glycerol in a chemically defined medium.

The present study first screened eight yeast strains from four genera for the ability to grow and accumulate lipids in chemically defined media containing glycerol as the sole carbon source. Two *Yarrowia lipolytica* strains which grew the best were further tested for growth, biomass yield, lipid production and fatty acid composition in the presence of different sources and concentrations of nitrogen and yeast extract.

MATERIAL AND METHODS

Microorganisms and inoculum preparation

Eight yeast strains belonging to species known for their propensity for oleaginicity were screened in this study. *Cryptococcus curvatus* DSM 70022, *Rhodotorula glutinis* DSM 10134, *Rhodotorula glutinis* DSM 70398, *Rhodosporidium toruloides* DSM 4444, and *Yarrowia lipolytica* DSM 70561 were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). *Cryptococcus albidus* NRC 3057 and *Rhodotorula gracilis* NRC 211008 were obtained from the National Research Council Canada Culture Collection, while *Yarrowia lipolytica* JDC 335 was kindly provided by Professor J.D. Cunningham, University of Guelph. The yeast strains were individually maintained at 4 ˚C on YPD agar plates containing per L: 10 g yeast extract, 20 g peptone, 20 g glucose, and 15 g agar and subcultured at monthly intervals.

For inoculum preparation, a loopful of cells from an isolated colony on YPD agar plate was aseptically transferred to 50 mL of liquid broth containing 1.67 g/L yeast nitrogen base without amino acids and ammonium sulfate (YNB w/o aa-AS), 10 g/L glucose and 5 g/L ammonium nitrate (NH_4NO_3) in a 125-mL Erlenmeyer flask. The culture was incubated at 28 ˚C with shaking at 180 rpm for 48 h.

Yeast screening

Two different screens were performed in chemically defined media to assess yeast growth and lipid production, respectively. In the first screen, the eight yeast strains were assessed individually for their ability to grow in 1.67 g/L YNB w/o aa-AS media containing 50, 100, 150, 200, 250, 300, 500 and 600 g/L glycerol plus 5 g/L NH_4NO_3. The cultures were grown in loosely capped test tubes (14 x125 mm) which were rotated about their vertical axis at 60 degrees from horizontal (Barbosa *et al.,* 1990) and 90 cycles per min at 28 ˚C for 96 h. A 0.05-mL aliquot of the inoculum culture was transferred to 10 mL of the medium contained in each test tube. Growth was monitored by direct measurement of the optical densities of the cultures against the medium blank at 600 nm (OD600) by using a Milton Roy Spectronic 20 spectrophotometer. Growth was judged to have occurred when more than 2 doublings of OD600 were observed.

In the second screen, yeast strains were assessed for both growth and lipid accumulation in 250-mL Erlenmeyer flasks. The medium consisted of 100 mL of 1.67 g/L YNB w/o aa-AS, 50 g/L glycerol (the concentration determined to be most suited for growth yield in the first screen) and 5 g/L NH_4NO_3 as nitrogen

source. Two mL of the inoculum culture were added to the medium and the mixture incubated at 28 °C with shaking at 180 rpm for 4 days. At daily intervals, samples were withdrawn for OD600 measurements by using a Ultrospec 3100 UV/Visible spectrophometer. Where necessary, samples were diluted prior to taking the OD600 measurements. On day 4, samples of the cells were removed for cell dry weight and lipid content determination. The two yeast strains, *Y. lipolytica* strains DSM 70561 and JDC 335, with the best growth and lipid accumulation potential were selected for further study.

Characterization of lipid accumulation by *Y. lipolytica* strains

The ability of *Y. lipolytica* strains to use 62.45 mM of either NH_4NO_3 (5 g/L) or urea (3.75 g/L) as the sole nitrogen source for growth was first examined in rotated test tubes as described above. Having verified that the strains grew better with urea than NH_4NO_3, urea was used in subsequent tests to examine the effect of varying concentrations of urea (ranging from 1 to 15 g/L resulting in C/N molar ratios ranging from 4.4 to 65.2) and concentrations of yeast extract (ranging from 2 to 10 g/L) on growth and lipid accumulation by the *Y. lipolytica* strains. For these experiments, 2 mL of the inoculum culture were placed in 100 mL of 1.67 g/L YNB w/o aa-AS, 50 g/L glycerol and varying concentrations of urea in 250-mL Erlenmeyer flasks and incubated at 28 °C with shaking at 180 rpm for 120 h.

After determining the optimal urea concentration, we tested the effect of adding yeast extract on growth and lipid accumulation. This was done by adding 2, 4, 6, 8 and 10 g/L yeast extract to 100 mL of 1.67 g/L YNB w/o aa-AS, 50 g/L glycerol and 1 g/L urea in 250-mL Erlenmeyer flasks. Duplicate samples were withdrawn periodically and analyzed for growth, cell dry weight, total cell lipids and residual glycerol concentrations.

For experiments to determine the fatty acid composition of the cellular lipids, *Y. lipolytica* DSM 70561 and JDC 335 cells were cultured in YNB w/o aa-AS media containing50 g/L glycerol and 1 g/L urea either with 2 g/L yeast extract or without. Cells were incubated as described above for 4 days and then collected for total cellular fatty acid analysis.

Analytical methods

For cell dry weight determination, 10 mL of the culture were centrifuged at 6,000xg for 10 min. The pellet was washed three times with deionized water and dried at 80 °C until constant weight (typically 24 h). The dry cell weight was determined gravimetrically.

Total lipids were extracted from whole yeast cells by the method of Folch *et al.* (1957) with some modifications and according to Salinee *et al.* (2012). Briefly, 30 mL of the cell culture were centrifuged at 6000xg for 15 min to collect yeast pellet. This pellet was washed twice with deionized water and the supernatant was discarded. To an aliquot of 100- to 1000-mg (wet weight) of the pellet was added 3.75 mL of chloroform/methanol (2:1, v/v) solution. The mixture was vortexed for 15 min at 22-24 °C. To this was added 1.25 mL of chloroform. The mixture was vortexed for 1 min followed by adding 1.25 mL of 1 M NaCl and the mixture vortexed again for 1 min. The mixture was centrifuged at 3000xg for 15 min to separate the aqueous and organic phases. The lower organic phase containing lipids was recovered with a Pasteur pipette and transferred to another glass tube. The organic solvent was evaporated under a stream of N_2 gas and the lipid weight was determined gravimetrically. The dry weight of total cell lipids was expressed as a percentage of total dry cell weight (Salinee *et al.*, 2012).

The total lipids were saponified and methyl esterified to yield fatty acid methyl esters which were analyzed using an Agilent 6890 GC-FID fitted with a capillary column (25.0 m x 200 µm x 0.33 µm, Ultra 2.5 % Phenyl Methyl Siloxane). The temperature of the injector and detector was 325 °C. Helium, at a flow rate of 1.2 mL/min, was used as the carrier gas. Fatty acid peaks were identified based on retention times in the GC elution profile. The peak area for each fatty acid methyl ester was quantified and the relative percentage of each fatty acid calculated based on its peak area relative to the total peak areas for all the fatty acids.

To determine the amount of residual glycerol, 2 mL of the liquid culture were centrifuged at 10,000xg for 10 min to remove the cell mass and other debris. The glycerol content in the supernatant was analyzed by HPLC using a Bio-Rad HPX-87H column operated at 40 °C (Lee *et al.,* 1986). The mobile phase was 0.005 M H_2SO_4 with a flow rate of 0.6 mL/min. Glucose served as the internal standard.

RESULTS AND DISCUSSION

Screening of yeasts for growth and lipid accumulation from glycerol

The eight yeast strains selected for screening belong to the genera *Cryptococcus*, *Rhodotorula*, *Rhodosporidium* and *Yarrowia* which are known to contain oleaginous strains able to accumulate high levels of cellular lipids (Pan *et al.,* 2009). The initial screening assessed how well these strains can grow in a chemically defined medium containing varying amounts of glycerol as the sole carbon source. Of the 8 yeasts tested, *C. albidus* NRC 3057 was unable to utilize glycerol as a sole carbon source. This was apparent in the inoculum preparation stage. On adding the dilute suspension of *C. albidus* NRC 3057 cells in the inoculum to the defined growth medium, no growth was observed for 4 days (Fig. 1A). Thus, *C. albidus* NRC 3057 was not tested further.

The other 7 yeast strains were able to grow in defined media containing glycerol as the sole carbon source. In general, growth decreased with increasing glycerol concentrations (Fig. 1B-H). The yeasts typically achieved the highest growth yields, based on OD600 measurements, at 50 g/L glycerol. Growth gradually decreased when glycerol concentration increased from 150 to 250 g/L (Fig. 1C-F). *Y. lipolytica* strains showed high growth yields up to 250 g/L glycerol. Above 250 g/L glycerol, very slight growth was observed by some of the cultures after 96 h (Fig. 1F-H). Among the seven yeasts tested, *Y. lipolytica* strains DSM 70561 and JDC 335 showed the highest growth yields at each glycerol concentration tested, followed by *R. glutinis* DSM 10134, *R. toruloides* DSM 4444, *C. curvatus* DSM 70022, *R. gracilis* NRC 211008, and *R. glutinis* DSM 70398 (Fig. 1).

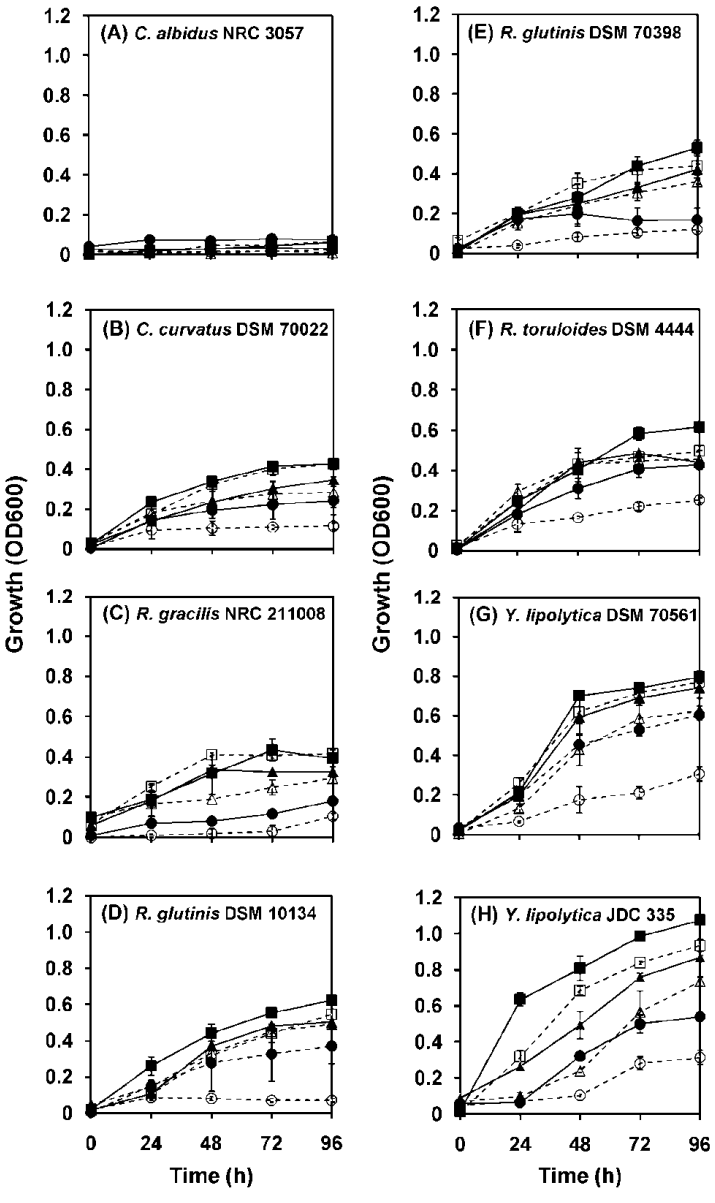

Figure 1 Growth of eight yeasts in chemically defined media supplemented with varying concentrations of glycerol. Symbols: (■) 50 g/L; (□) 100 g/L; (▲) 150 g/L; (△) 200 g/L; (●) 250 g/L; and (○) 300 g/L glycerol. Each value is the mean±SEM for 3 independent experiments

Some authors have examined the effect of varying glycerol concentration on growth of oleaginous yeasts. However, these studies used lower concentrations of glycerol than the maximum concentration that we have used here. As an example, Papanikolaou *et al.* (2008) examined the biomass yield of *Y. lipolytica* strain ACA-DC 50109 growing on glycerol concentrations ranging from 20–164 g/L; no differences were observed. The growth inhibitory effect of high glycerol concentrations has been reported in other oleaginous microorganisms. For

example, growth rate of *Cryptococcus curvatus* ATCC 20509 cells decreased when glycerol concentration increased from 32 to 128 g/L (Meesters *et al.,* 1996). The oleaginous alga *Schizochytrium limacinum* SR21 could grow only in the presence of low concentrations (25 and 35 g/L) of glycerol. Growth was inhibited at higher glycerol concentrations (60 to 100 g/L) (Liang *et al.,* 2010). Pyle *et al.* (2008) and Chi *et al.* (2007) also reported that the optimum glycerol concentrations for maximal biomass yield of *S. limacinum* were 40 g/L and 64–85 g/L, respectively.

The seven yeast strains were next screened for growth and lipid accumulation in Erlenmeyer flasks each containing 100 mL of defined media supplemented with 50 g/L glycerol, the concentration found in the first screen to give the best growth yields for the yeasts. The results showed that *Y. lipolytica* strains JDC 335 and DSM 70561 grew better than others, reaching OD600 values of 5.56 and 5.16, respectively, in the first 48 h. *R. toruloides* DSM 4444 grew after 24 h while *C. curvatus* DSM 70022, *R. gracilis* NRC 211008, *R. glutinis* DSM 10139 and *R. glutinis* DSM 70398 grew after a lag phase of 48 h (Fig. 2A). The lag periods observed were similar to the first screen. The amounts of lipids accumulated at 96 h by *Y. lipolytica* strains DSM 70561 and JDC 335 were 25.75 ± 0.75 % and 22.06 ± 5.80 % (w/w, % dry weight of lipid/cell dry weight), respectively (Fig. 2B). The lipid content of the other five yeasts ranged from 7–17 % (w/w), respectively, over the same time period. Based on their rapid growth, high growth yields and high lipid accumulation in defined media with glycerol as the sole carbon source, the two *Y. lipolytica* strains were selected for further studies.

Effect of varying concentrations of urea and yeast extract on biomass and lipid accumulation by *Y. lipolytica* strains DSM 70561 and JDC 335

Yeast cell growth is influenced by the amount of nitrogen, usually provided as ammonium (NH_4^+) or urea in the medium. In the next phase of the study, the use of either urea or NH_4NO_3 as the nitrogen source on growth of the two *Y. lipolytica* strains was compared. These studies were done in rotated test tubes, each containing 10 mL of YNB w/o aa-AS supplemented with 50 g/L glycerol plus either 62.45 mM NH_4NO_3 or urea. The two yeast strains grew fairly well with either urea or NH_4NO_3 as the nitrogen source. The OD600 values of DSM 70561 and JDC 335 when grown for 96 h in NH_4NO_3 and urea were 0.78 and 1.31, and 1.03 and 1.53, respectively. Since the yeasts grew to significantly higher OD values in urea, we used urea as the N source in subsequent studies.

We next investigated the effect of varying urea concentrations on lipid accumulation by the two *Y. lipolytica* strains in Erlenmeyer flasks. Biomass yields of DSM 70561 and JDC 335 at 120 h ranged from 2.91 to 3.54 g/L and 2.85 to 3.56 g/L, respectively (Table 1). These strains consumed about 21 to 25 g/L of glycerol, respectively, out of the initial concentration of 50 g/L. The total lipid content of DSM 70561 and JDC 335 cells gradually decreased with increasing urea concentrations. The maximum lipid accumulated was found in the presence of 1 g/L urea; the lipid contents of DSM 70561 and JDC 335 were 35.54 ± 2.33 % and 46.16 ± 2.89 % (w/w, dry weight basis), respectively. Other researchers have also reported urea as an excellent nitrogen source for lipid production in yeasts, such as *Rhodotorula glutinis* (Saenge *et al.,* 2011) and *Trichosporon fermentans* (Zhu *et al.,* 2008). Although the *Y. lipolytica* strains accumulated high levels of lipids, the amount of glycerol consumed was limited in the chemically defined medium with urea as the nitrogen source. Thus, we tested if yeast extract supplementation, ranging from 2 to 10 g/L, might stimulate growth, increase glycerol consumption and also accumulate more biomass and lipids by the yeasts. Cells of *Y. lipolytica* DSM 70561 and JDC 335 produced more biomass and lipids when supplemented with yeast extract as compared to growth in the absence of yeast extract (Tables 1 and 2). Cell dry weight yields by strains DSM 70561 and JDC 335 at 120 h ranged from 4.27 to 4.59 g/L and 4.01 to 4.75 g/L, respectively. The amount of glycerol consumed by the two yeasts averaged 20 to 23 g/L. This level of consumption was similar to those seen in the absence of yeast extract. The higher biomass yield was most likely due to the yeast extract supplementation, although there was no apparent trend towards greater biomass yield with increasing yeast extract concentrations. The highest amounts of lipid accumulated, 37.1 % and 54.4 % (w/w) by DSM 70561 and JDC 335, respectively, were observed at the lowest yeast extract supplementation of 2 g/L. At higher yeast extract concentrations (4 to 10 g/L), while similar cell biomass was accumulated, glycerol consumption was not substantially increased. Yeast extract supplementation has been reported to increase biomass (51.8 g/L, w/v) and lipid content (65.1%, w/w) in *C. curvatus* O3 (Zhang *et al.,* 2011). In another study with the oleaginous alga *Chlorella protothecoides*, supplementation of the medium with 1, 4, 7, and 10 g/L of yeast extract led to biomass yields of 9.05, 17.99, 18.6, and 19.81 g/L, respectively; however, the lipid content decreased significantly from 53.2 %, 46.0 %, 21.5 % to 18.9 % (w/w), respectively (Xiong *et al.,* 2008).

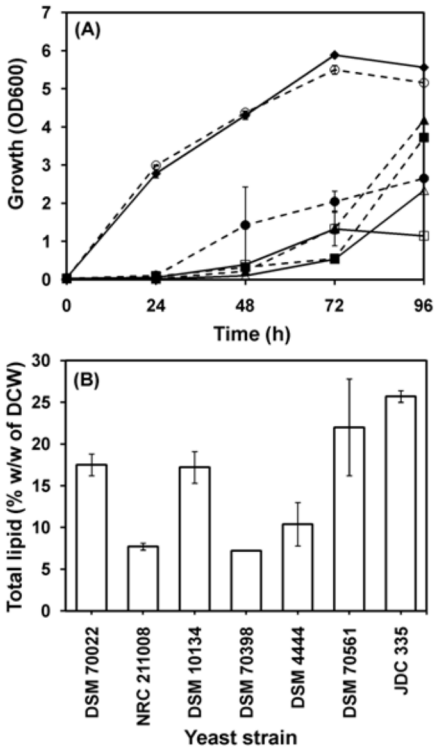

Figure 2 Growth profiles of seven yeasts (Panel A) and their lipid content (Panel B). Cultures were grown in YNB w/o aa-AS medium with 50 g/L glycerol and 5 g/L NH_4NO_3. Symbols: (■) *C. curvatus* DSM 70022; (□) *R. gracilis* NRC 211008; (▲) *R. glutinis* DSM 10134; (△) *R. glutinis* DSM 70398; (●) *R. toruloides* DSM 4444; (○) *Y. lipolytica* DSM 70561; and (◆) *Y. lipolytica* JDC 335. DCW = dry cell weight.

Table 1 Dry cell weight (DCW), lipid content and glycerol consumption in *Y. lipolytica* DSM 70561 and *Y. lipolytica* JDC 335 when cultured for 120 h in YNB w/o aa-AS media containing 50 g/L glycerol with varying concentrations of urea

Yeast strains	Urea concentration (g/L)	DCW (g/L)	Lipid content (wt %)	Glycerol consumption (g/L)
Y. lipolytica DSM 70561	1	3.54±0.39	35.54±2.33^a	23.33±0.28^a
	2	3.32±0.41	35.29±3.60^a	20.47±0.26^a
	4	3.54±0.53	27.18±3.15^b	21.60±0.43^a
	7	2.91±0.13	21.54±1.62^b	26.67±0.91^b
	10	2.92±0.12	22.64±3.77^b	27.37±0.84^b
	15	3.13±0.02	17.40±2.24^c	28.87±0.70^b
Y. lipolytica JDC 335	1	3.56±0.55	46.16±2.89^a	21.87±0.46^a
	2	3.35±0.21	42.43±2.60^a	22.93±0.91^a
	4	2.85±0.14	46.01±4.81^a	26.20±0.95^b
	7	3.23±0.17	37.08±4.16^b	16.63±0.06^c
	10	2.92±0.29	43.41±3.61^a	21.13±0.44^a
	15	3.08±0.10	37.58±3.20^b	15.90±0.46^c

Values are means±SEM. Control: the culture without addition of nitrogen source. Different letters in the same column indicate significant treatment difference ($P < 0.05$).

Table 2 Dry cell weight (DCW), lipid content, and glycerol consumption in *Y. lipolytica* DSM 70561 and *Y. lipolytica* JDC 335 when cultured for 120 h in YNB w/o aa-AS media containing 50 g/L glycerol plus 1 g/L urea with varying concentrations of yeast extract

Yeast strains	Yeast extract concentration (g/L)	DCM (g/L)	Lipid content (wt %)	Glycerol consumption (g/L)
Y. lipolytica DSM 70561	2	4.27±0.55	37.09±2.84[a]	22.83±0.28[a]
	4	4.42±1.02	29.84±1.35[b]	19.73±0.25[bc]
	6	4.32±0.71	34.59±6.45[a]	20.27±0.11[b]
	8	4.56±0.81	31.10±5.01[b]	19.10±0.34[c]
	10	4.59±0.65	36.03±2.32[a]	20.37±0.19[b]
Y. lipolytica JDC 335	2	4.24±0.47	54.44±1.01[a]	24.70±0.15[a]
	4	4.21±0.59	45.59±1.27[b]	20.50±0.14[b]
	6	4.75±0.85	47.76±3.67[b]	21.13±0.13[b]
	8	4.01±0.35	42.44±8.53[b]	25.03±0.38[a]
	10	4.66±0.43	46.36±5.09[b]	22.33±0.30[b]

Values are means±SEM. Control: the culture without addition of nitrogen source. Different letters in the same column indicate significant treatment difference ($P < 0.05$).

Fatty acid composition of *Y. lipolytica* DSM 70561 and JDC 335 cells

The predominant fatty acids in lipids isolated from strains DSM 70561 and JDC 335 grown in the absence of yeast extract consisted of C16:1 [5.7 % and 5.0 % (w/w), respectively], C16:0 [17.7 % and 16.7 % (w/w), respectively], C18:1+C18:2 [49.1 % and 51.9 % (w/w), respectively] and C18:0 [19.0 % and 20.6 % (w/w), respectively] (Table 3). When cells were grown in the presence of yeast extract, the same predominant fatty acids were found, albeit in slightly different proportions (Table 3). Among the C16 fatty acids, the concentration of the saturated C16:0 fatty acid was about three times higher than that of mono-unsaturated C16:1 fatty acid. The other fatty acids accounted for less than 5 % of the total cellular fatty acids. The ratio of saturated vs unsaturated fatty acids was 0.7 for both strains regardless of whether cells were grown with or without yeast extract (Table 3). In an earlier study, Papanikolaou and Aggelis (2002) reported high percentages of C18:1 (47 %, w/w) and lower amounts of C16:1 (15 %, w/w) and C18:0 (13 %, w/w) among the cellular lipid accumulated by *Y. lipolytica* LGAM S(7)1 cells grown on 50 g/L glycerol. In another study, C18:1 was reported to be the predominant fatty acid (44.9 %, w/w) accumulated by *Y. lipolytica* W29 cells grown on glucose (Beopoulos *et al.,* 2008). It is well known that the media and cultivation conditions can markedly influence the fatty acid composition of yeast cells.

Table 3 Percentages of total fatty acids from *Y. lipolytica* strains DSM 70561 and JDC 335 grown in YNB w/o aa-AS media containing 50 g/L glycerol plus 1 g/L urea with and without yeast extract

Yeast strain	Fatty acid	Percent of total fatty acid (%)	
		with yeast extract	without yeast extract
Y. lipolytica DSM 70561	C14:0	0.3±0.04	0.3±0.04
	C15:1	0.5±0.01	0.9±0.03
	C16:1	6.2±0.17	5.7±0.13
	C16:0	16.4±0.37	17.7±0.17
	C17:1	1.2±0.04	0.8±0.02
	C17:0	0.4±0.03	0.6±0.08
	C18:1+C18:2	49.1±1.82	51.6±1.32
	C18:0	22.0±0.15	19.0±1.14
	C19:1	0.1±0.01	0.4±0.02
	C20:0	1.1±0.02	0.8±0.06
	SAT/UNSAT[a]	0.7	0.7
Y. lipolytica JDC 335	C14:0	0.3±0.03	0.3±0.03
	C15:1	0.3±0.02	1.0±0.02
	C15:0	0.8±0.08	0.7±0.04
	C16:1	8.2±0.07	5.0±0.05
	C16:0	17.4±1.06	16.7±0.31
	C17:1	1.4±0.07	0.8±0.03
	C17:0	0.4±0.05	0.2±0.02
	C18:1+C18:2	46.7±0.28	51.9±0.37
	C18:0	21.7±0.02	20.6±0.23
	C19:1	1.0±0.02	0.8±0.01
	C20:0	0.8±0.02	1.1±0.02
	SAT/UNSAT[a]	0.7	0.7

Values are means±SEM.
[a]SAT = saturated fatty acids; UNSAT = unsaturated fatty acids.

Interestingly, palm oil (Moser, 2008) which are used as biodiesel feedstocks also contain moderate to high levels of C18:1, C18:2, C18:0 and C16:0 fatty acids. In our study, the main fatty acids of *Y. lipolytica* DSM 70561 and JDC 335 were C16:0, C16:1, C18:1, C18:2 and C18:0, and these should be suitable for conversion to biodiesel.

CONCLUSION

Many researchers have studied lipid accumulation in *Y lipolytica* strains, to our knowledge *Y. lipolytica* strain DSM 70561 and JDC 335 showed remarkable growth on glycerol used as sole carbon source and grew best in the presence 1 g/L urea as nitrogen source. The biomass yield increased with the addition yeast extract in media. However, the amount of lipids accumulation was not affected, by *Y. lipolytica* DSM 70561 and JDC 335 accumulated 37.1 % and 54.4 % lipid in their cells, respectively. The accumulated lipids were predominantly saturated and mono-unsaturated species of C16 and C18 fatty acids. *Y. lipolytica* strains DSM 70561 and JDC 335 exhibited several desirable characteristics for an industrial use in recycling crude glycerol into useful fatty acids.

Acknowledgments: This research was supported by a grant from the University Staff Development Program of the Commission on Higher Education (Thailand) and partially by the NSERC Bioconversion Network (Canada).

REFERENCES

ASAD-UR-REHMAN, MATSUMURA, M., NOMURA, N., SATO, S. 2008. Growth and 1, 3-propanediol production on pre-treated sunflower oil bio-diesel raw glycerol using a strict anaerobe-*Clostridium butyricum*.*Current Research of Bacteriology,* 1(1), 7-16.

BARBOSA, M. De F.S., LEE, H., COLLINS-THOMPSON, D. L. 1990. Additive effects of alcohols, their acidic by-products, and temperature on the yeast *Pachysolen tannophilus*.*Applied Environmental Microbiology,* 56(2), 545-550.

BEOPOULOS, A., - MROZOVA, Z., - THEVENIEAU, F., DALL, M., - HAPALA, I., PAPANIKOLAOU, S., CHARDOT, T., NICAUD, J. 2008. Control of lipid accumulation in the yeast *Yarrowia lipolytica*.*Applied Microbiology and Biotechnology,* 74(24), 7779–7789.

CHI, Z. P., PYLE, D., WEN, Z., FREAR, C, CHEN, S. 2007. A laboratory study of producing docosahexaenoic acid from biodiesel-waste glycerol by microalgal fermentation.*Process Biochemistry,* 42(11), 1537–1545.

DHARMADI, Y., MURARKA, A., GONZALEZ, R. 2006. Anaerobic fermentation of glycerol by *Escherichia coli*: a new platform for metabolic engineering.*Biotechnology and Bioengineering,* 94(5), 821–829.

EASTERLING, E. R., TODD FRENCH, W., HEMANDEZ, R., LICHA, M. .2009. The effect of glycerol as a sole and secondary substrate on the growth and fatty acid composition of *Rhodotorula glutinis*.*Bioresource Technology,* 100(1), 356–361.

FOLCH, J., LEES, M., SLOANE STANLEY, G. H. 1957. A simple method for the isolation and purification of total lipid from animal tissues.*Journal of Biology and Chemistry,* 226(1), 497-509.

GONZA´LEZ-PAJUELO, M., Andrade, J. C., Vasconcelos, I. 2004. Production of 1,3-propanediol by *Clostridium butyricum* VPI 3266 using a synthetic medium and raw glycerol.*Journal of Industrial Microbiology and Biotechnology,* 31(9), 442–446.

ITO, T., NAKASHIMADA, Y., SENBA, K., MATSUI, T. - NISHIO, N. 2005. Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process.*Journal of Bioscience and Bioengineering,* 100(3), 260–265.

LEE, H., BIELY, P., LATTA, K. - BARBOSA, M. F. S., SCHNEIDER, H. 1986. Utilization of xylan by yeasts and its conversion to ethanol by *Pichia stipitis* strains.*Applid Environmental Microbiology,* 52(2), 320-324.

LI, W., DU, W., LI, Y. H., LIU, D. H., ZHAO, Z. B. 2007. Enzymatic transesterification of yeast oil for biodiesel fuel production.*Chinese Journal of Process Engineering,* 7(4), 137–140.

LIANG, Y., NICOLAS, S., CUI, Y., BLACKBUM, J. W. 2010. Batch stage study of lipid production from crude glycerol derived from yellow grease or animal fats through microalgal fermentation.*Bioresource Technology,* 101(7), 6745–6750.

MEESTERS, P. A. E. P., HUIJBERTS, G. N. M., EGGINK, G. 1996. High-cell-density cultivation of the lipid accumulating yeast *Cryptococcus curvatus* using glycerol as a carbon source.*Applid Environmental Microbiology,* 45(5), 575-579.

NARAYAN, G. P., MANOJ, K., VATCHRAVELU, N., BHAGYALAKSHMI, N., MAHADEVASWAMY, M. 2005. Utilization of glycerol as carbon source on the growth, pigment and lipid production in *Spirulina platensis*.*International Journal of Food Sciences and Nutrition,* 56(7), 521–528.

PAN, L. X., YANG, D. F., SHAO, L., LI W., CHEN, G. G., LIANG, Z. Q. 2009. Isolation of the oleaginous yeasts from the soil and studies of their lipid-producing capacities.*Food Technology and Biotechnology,* 47(2), 215–220.

PAPANIKOLAOU, S., AGGELIS, G. 2002. Lipid production by *Yarrowia ipolytica* growing on industrial glycerol in a single-stage continuous culture.*Bioresource Technoogy,* 82(1), 43–49.

PAPANIKOLAOU, S., CHATZIFRAGKOU, A., FAKAS, S., GALIOTOU-PANAYOTOU, M., KOMAITIS, M., NICAUD, J., AGGELIS, G. 2009. Biosynthesis of lipids and organic acids by *Yarrowia lipolytica* strains cultivated on glucose.*European Journal of Lipid Science and. Technoogy,* 111(12), 1-12.

PAPANIKOLAOU, S., MUNIGLIA, L., CHEVALOT, I., AGGELIS, G., IVAN, M. 2003. Accumulation of a cocoa-butter-like lipid by *Yarrowia lipolytica* cultivated on agro-industrial residues.*Current Microbiology,* 46(2), 124–130.

PAPANIKOLAOU, S., FAKAS, S., FICK, M., CHEVALOT, I., GALIOTOU-PANAYOTOU, M., KOMAITIS, M., 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 and Bioenergimeering,* 32(1), 60-71.

PYLE, D. J., GARCIA, R. A, WEN, Z. Y. 2008. Producing docosahexaenoic acid (DHA)-rich algae from biodiesel-derived crude glycerol: effects of impurities on DHA production and algal biomass composition.*Journal of Agricultural of Food Chemistry,* 56(11), 3933–3939.

RATLEDGE, C. 1991. Microorganisms for lipids.*Acta Biotechnology,* 11(5), 429-438.

RATLEDGE, C. 2004. Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochemie,* 86(11), 807-815.

RYMOWICZ, W., RYWINSKA, A., ZAROWSKA, B., JUSZCZYK, P. 2006. Citric acid production from raw glycerol by acetate mutants of *Yarrowia lipolytica*.*Chemical Papers,* 60(5), 391–394.

SRIWONGCHAI, S., POKETHITIYOOK, P., PUGKAEW, W., KRUATRACHUE, M., LEE, H. 2012. Optimization of lipid production in the oleaginous bacterium *Rhodococcus erythropolis*growing on glycerol as the sole carbon source.*African Journal of Biotectonology,* 11(79), 14440-14447.

SAENG, C., CHEIRSLIP, B., SUKSAROGE, T. T., BOURTOOM, T. 2011. Efficient concomitant production of lipids and carotenoids by oleaginous red yeast *Rhodotorula glutinis* cultured in palm oil mill effluent and application of lipids for biodiesel production.*Biotechnolology and Bioprocess Engineering,* 16(1) 23-33.

XIONG, W., LI, X., XIANG, J., WU, Q. 2008. High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production.*Applid Environmental Microbiology,* 78(1), 29 - 36.

ZHANG, J., FANG, X., ZHU, X. L., LI, Y., XU, H. P. - ZHAO, B. F., CHEN, L., ZHANG, X. D. 2011. Microbial lipid production by the oleaginous yeast *Cryptococcus curvatus* O3 grown in fed-batch culture.*Biomass and Bioenineering,* 35(5), 1906-1911.

ZHU, L. Y., ZONG, M. H., WU, H. 2008. Efficient lipid production with *Trichosporon fermentans* and its use for biodiesel preparation.*Bioresource Technololgy,* 99(16), 7881–7885.